The Dissolution Analysis of Sulfadoxine/Pyrimethamine Combination Tablets

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This dissertation is dedicated to Frans, Alma and Izet Badenhorst

ABSTRACT

Disease is as old as life itself and the treatment thereof ancient too. A disease that still claims over a million lives annually and is considered a health problem in approximately 90 countries is malaria, even though it was discovered over a hundred years ago. Malaria has however been treated successfully with numerous anti-malarial drugs such as Fansidar®.

Fansidar® contains 500 mg sulfadoxine and 25 mg pyrimethamine and is used as second line treatment for malaria. Research has shown that African children can be protected against malaria by means of prophylaxis. In Gambia children were treated with a pyrimethamine and dapsone combination and the mortality decreased by 35% while in Malawi the sulfadoxine-pyrimethamine combination administered to pregnant women reduced placental malaria by 72%. However, for any pharmaceutical product to be effective in the treatment of disease, it must be thoroughly tested and submitted to the various standards to prove the safety and efficacy of such a product. These tests and standards are set in international pharmacopoeias and a product must comply with the acceptance criteria for that particular product.

During this study the emphasis fell on the dissolution test of the sulfadoxine-pyrimethamine combination tablet as stipulated by the USP. The pyrimethamine component constantly fails to comply with the dissolution requirements and it was decided that an alternative dissolution medium should be considered, which set the aim of this study. Furthermore it was suspected that sulfadoxine impurities interfered with the pyrimethamine peak in the HPLC chromatogram, as it appeared to produce a false positive peak.

For this purpose the USP method was used as a foundation to develop an analytical method with a more alkaline mobile phase by adjusting the pH. Because of this, the pyrimethamine peak was forced to appear after the sulfadoxine peak, preventing the impurities of the latter to affect the pyrimethamine peak. The results obtained from the modified analytical method was compared to that of the original analytical method and proved to be the better of the two as it produced best results. Three different media were tested with the new as well as the original analytical method and although the overall % RSD obtained for sulfadoxine didn't vary much for the two methods, pyrimethamine produced a much smaller % RSD with the new method.

With the new method as method of choice, dissolution tests of the innovator product (Fansidar®) and a generic (Falcistat®) were performed in three media (PBS pH 6.8, 0.1 N HCl and water). For both Fansidar® and Falcistat® 0.1 N HCl produced the best results as this was the only

medium in which both active ingredients complied with the dissolution criteria, hence 0.1 N HCl was considered the medium of choice.

Hereafter, stability test dissolutions were performed on both Fansidar® and Falcistat® to ensure the stability of sulfadoxine and pyrimethamine in the medium. The two concentrations used for these dissolution tests were 0.01 N and 0.1 N HCl. The concentrations obtained for pyrimethamine didn't differ significantly however less then 50% sulfadoxine dissolved in 0.01 N HCl for both Fansidar® and Falcistat®. Even though the stability didn't deteriorate remarkably in either of the two cases, 0.1 N HCl was still considered the medium of choice as it produced the best results.

Other generic products were tested to confirm the findings. Using 0.1 N HCl as dissolution medium and the newly developed method, dissolution tests were performed on the seven generics and the results proved that the modified method was indeed the method of choice as was the case with 0.1 N HCl as dissolution medium. All the products complied with the dissolution criteria for sulfadoxine and pyrimethamine except for Dionsdar® (sulfadoxine failed to comply) and Tansidar® (both active ingredients failed to comply). It was decided to perform another dissolution test on Tansidar® using PBS as dissolution medium (USP dissolution medium). Again Tansidar® failed to comply with the dissolution criteria as sulfadoxine produced worse results with the second dissolution test whilst pyrimethamine didn't dissolve. It is suspected that the composition of the Tansidar® tablets might be in question.

To conclude, it is believed that the success of 0.1 N HCl as dissolution medium in this study is due to the fact that it is the USP dissolution medium for pyrimethamine single component tablets. Hence, it would be the ideal solvent in this case as pyrimethamine doesn't dissolve easily or at all in most solvents whilst sulfadoxine wasn't negatively affected by the change in dissolution medium. Furthermore, the results obtained for the modified method were more accurate and of better quality as no impurities interfered with the small pyrimethamine peak.

OPSOMMING

Siekte en die behandeling daarvan is so oud soos die lewe self. 'n Siekte wat nog steeds meer as 'n miljoen lewens jaarliks eis en wat as 'n gesondheidsprobleem beskou word in ongeveer 90 lande, is malaria, alhoewel dit al meer as 'n honderd jaar gelede ontdek is. Malaria is en word steeds suksesvol met verskeie anti-malariamiddels soos Fansidar® behandel.

Fansidar® bevat 500 mg sulfadoksien en 25 mg pirimetamien en word as tweedelinie behandeling vir malaria gebruik. Navorsing het bewys dat kinders in Afrika teen malaria beskerm kan word deur middel van profilakse. In Gambië is kinders met 'n kombinasie van pirimetamien en dapsoon behandel wat aanleiding gegee het tot 'n 35% afname in mortaliteit. In Malawi is die sulfadoksien-pirimetamien kombinasie aan swanger vrouens toegedien wat tot 'n afname van 72% in plasentale malaria gelei het. Om 'n siekte egter effektief te behandel, moet alle farmaseutiese produkte deeglik getoets word en aan verskeie standaarde voldoen om die veiligheid en effektiwiteit van so 'n produk te verseker. Hierdie toetse en standaarde word in internasionale farmakopieë uiteengesit en 'n produk moet aan die aanvaardingskriteria vir die spesifieke produk voldoen.

In die studie het die klem op die dissolusietoets van sulfadoksien-pirimetamien kombinasie tablet, soos uiteengesit in die USP, geval. Die pirimetamienkomponent voldoen voortdurend nie aan die dissolusievereistes nie en die doel van hierdie studie was om 'n alternatiewe dissolusiemedium te ondersoek. Dit is ook vermoed dat onsuiwerhede, afkomstig van sulfadoksien, steurnisse met die pirimetamienpiek in die HDVC-chromatogram veroorsaak, siende dat 'n vals positiewe piek verkry is.

Om hierdie rede is die USP-metode as grondslag gebruik om 'n analitiese metode te ontwikkel met 'n meer alkaliese mobiele fase deur verandering in pH. Die verandering in pH het 'n verskuiwing van die pirimetamienpiek tot gevolg gehad en die pirimetamienpiek het nou ná die sulfadoksienpiek geëlueer wat verhoed dat enige onsuiwerhede van die sulfadoksienpiek die pirimetamienpiek nadelig sou beïnvloed. In vergelyking met die oorspronklike analitiese metode het die aangepasde analitiese metode beter resultate gelewer. Drie verskillende media is met beide die nuwe en die oorspronklike analitiese metode getoets en alhoewel die algehele relatiewe standaardafwyking (% RSA) vir sulfadoksien vir beide metodes nie veel verskil het nie, het pirimetamien met die aangepasde metode 'n heelwat kleiner % RSA verkry.

Met die nuwe metode as voorkeurmetode, is dissolusietoetse op die innoveerder produk (Fansidar®) en 'n generiese produk (Falcistat®) in drie verskillende media (PBS pH 6.8, 0.1 N

HCl en water) uitgevoer. Met beide Fansidar® en Falcistat® het 0.1 N HCl die beste resultate gelewer en dit was ook die enigste medium waarin beide aktiewe bestanddele daarin geslaag het om aan die dissolusiekriteria te voldoen. Om hierdie rede word 0.1 N HCl as die voorkeur-medium beskou.

Stabiliteitstoetsdissolusies is hierna op beide Fansidar® en Falcistat® uitgevoer om die stabiliteit van sulfadoksien en pirimetamien in die medium te verseker. Die twee konsentrasies wat vir die dissolusietoetse gebruik was, was 0.01 N en 0.1 N HCl. Die konsentrasies wat vir pirimetamien verkry is, het nie 'n merkwaardige verskil getoon nie, terwyl minder as 50% sulfadoksien in 0.01 N HCl opgelos het vir Fansidar® en Falcistat®. Hoewel die stabiliteit van beide aktiewe bestanddele nie merkwaardig afgeneem het nie, bly 0.1 N HCl die voorkeurmedium, siende dat die beste resultate met dié medium verkry is.

Ander generiese produkte is ook getoets om die bevindinge te bevestig. Dissolusietoetse is op sewe generiese produkte uitgevoer deur 0.1 N HCl as dissolusiemedium en die nuut ontwikkelde metode te gebruik. Die resultate het weer bewys dat die aangepasde metode die metode van voorkeur is, asook die gebruik van 0.1 N HCl as dissolusiemedium. Al die produkte het voldoen aan die dissolusiekriteria vir sulfadoksien en pirimetamien behalwe Dionsdar® (sulfadoksien het nie voldoen nie) en Tansidar® (beide aktiewe bestandele het nie voldoen nie). Daar is besluit om nog 'n dissolusietoets op Tansidar® uit te voer met PBS as dissolusiemedium (USP-dissolusiemedium). Weer het Tansidar® nie aan die dissolusiekriteria voldoen nie, sulfadoksien het swakker resultate gelewer met die tweede dissolusietoets terwyl pirimetamien nie opgelos het nie. Die vermoede is dat Tansidar® se samestelling bevraagteken word.

Ten slotte word geglo dat 0.1 N HCl se sukses as dissolusiemedium in hierdie studie toe te skryf is aan die feit dat dit die USP-dissolusiemedium vir tablette met pirimetamien as enkele komponent is. Dus sal dit die ideale oplosmiddel in dié geval wees, siende dat pirimetamien moeilik oplosbaar is, terwyl sulfadoksien geensins negatief deur die verandering in dissolusiemedium beïnvloed is nie. Die resultate van die aangepasde metode was meer akkuraat en van beter gehalte omrede geen onsuiwerhede met die klein pirimetamienpiek gesteur het nie.

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ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
AUC	area under curve
Av.	average
FDB	Food and Drug Board
HPLC	high pressure liquid chromatography
HDVC	hoë druk vloeistof chromatografie
HIV	human immunodeficiency virus
ICH	International Conference on Harmonisation
PABA	para-aminobenzoic acid
% RSD	percentage relative standard deviation
% RSA	persentasie relatiewe standaardafwyking
PBS	phosphate buffer solution
P. falciparum	*Plasmodium falciparum*
SANAS	South African National Accreditation System
SD	standard deviation
TB	tuberculosis
USP	United States Pharmacopoeia
USP/DQI	United States Pharmacopoeia/Drug Quality and Information Program
WHO	World Health Organization

CHAPTER 1

INTRODUCTION AND AIM

1.1 Introduction

One of the main causes of death and disease today is malaria, especially in numerous parts of Asia, sub-Saharan Africa and the Americas. Of the four *Plasmodium* species that cause malaria, *Plasmodium falciparum* is responsible for the majority of illness and death in humankind (Duraisingh & Refour, 2005; Idro *et al.*, 2005; Okie, 2005; Worrall *et al.*, 2005). In sub-Saharan Africa this disease has a profound impact on children and infants, whilst millions have already died from AIDS (Acquired Immunodeficiency Syndrome) over 24 million people are infected with HIV-1 (Human Immunodeficiency Virus) (Esparza, 2005; Harms & Feldmeier, 2005). In addition to this, malaria adds in mortality whilst the spread of chloroquine resistant strains of the *plasmodium* parasites across Africa increases (Farooq & Mahajan, 2004; Mahajan *et al.*, 2005). Approximately 3 million people, of whom more than half are children, die of malaria caused by *P. falciparum* annually. Mortality and morbidity increases every year with over 500 million people infected with *P. falciparum*, presenting clinical symptoms of mild to severe malaria.

There exist several reasons for the increase in the occurrence of malaria including:

1. an increase of the protozoan parasite's resistance to anti-malarial drugs,
2. the development of the Anopheles mosquito vectors' resistance to numerous insecticides and
3. the growth and the widespread migration of vulnerable populations to vastly endemic areas (Abdel-Hameed, 2003; Gregson & Plowe, 2005).

Malaria can be treated with various anti-malarial drugs. Sulfadoxine and pyrimethamine combination therapy was introduced into clinical practice for the prevention and treatment of malaria in the late 1960s as a follow-up on the drug chloroquine.

The first product by the name of Fansidar® containing sulfadoxine and pyrimethamine in combination was produced by Roche in 1971. Since the expiration of the Fansidar® patent, numerous generic products have been produced worldwide, contributing towards cheaper anti-malaria therapy.

Since the introduction of sulfadoxine and pyrimethamine therapy, parasite resistance to sulfadoxine and pyrimethamine has been documented in various reports.

It is a concern that there might be a link between the low quality of sulfadoxine and pyrimethamine products and the occurrence of parasite resistance to these products. This may be due to the fact that the malaria parasite is subjected to sub-therapeutic dose of sulfadoxine and pyrimethamine as a result of low quality products.

This initiated a quality screening project by the World Health Organization (WHO) (World Health Organization, 2004). Various brands of sulfadoxine and pyrimethamine combination products from various African countries were subjected to quality screening in terms of content and dissolution characteristics.

From this study it was noted that many products did not comply with the dissolution criteria of the United States Pharmacopoeia (USP) in terms or the pyrimethamine component. This phenomenon was also observed by Kayumba (Kayumba *et al.*, 2004). The fact that the sulfadoxine component of the pyrimethamine-failing products complied with the dissolution criteria, suggests that pyrimethamine failing to dissolve during the dissolution test may not be due to bad formulation but rather to limitations within the USP dissolution test for pyrimethamine combination products.

It was anticipated that the phenomena may be due to the fact that the USP dissolution medium prescribed for sulfadoxine and pyrimethamine products is not suitable in the sense that it may be too discriminating when compared to the dissolution medium required by the USP for the dissolution testing of products containing only pyrimethamine.

In order to investigate this phenomenon the following objectives for the study were set:

1.2 Objectives

- To investigate and recommend a dissolution medium that is more suitable in terms of discriminating power than the dissolution medium of the USP currently prescribed for the dissolution testing of sulfadoxine and pyrimethamine combination products.
- To amend the high pressure liquid chromatography (HPLC) method utilized by the USP for the analysis of dissolution samples of sulfadoxine and pyrimethamine combination products, in order to enhance the robustness and selectivity of the HPLC method.
- To investigate the chemical stability of the dissolution samples of sulfadoxine and pyrimethamine combination products, as a function of dissolution medium and analytical run time.

CHAPTER 2

MALARIA AND ITS TREATMENT

2.1 Malaria

2.1.1 A health problem

Of all the communicable illnesses, Malaria, known as the globe's greatest tropical parasitic infection, claims the most lives aside from tuberculosis (TB). Being a health problem in over 90 countries and inhabited by approximately 2400 million people, an expected 300 – 500 million clinical cases occur per annum and more than 1 million lives are claimed yearly (Greenwood *et al.*, 2005). In the year 2001, the three illnesses TB, HIV and malaria combined killed approximately 5.7 million people, of which the majority was young children together with men and women of their productive years, and in developing countries, deemed liable for 23% of fatalities (Theobald *et al.*, 2006).

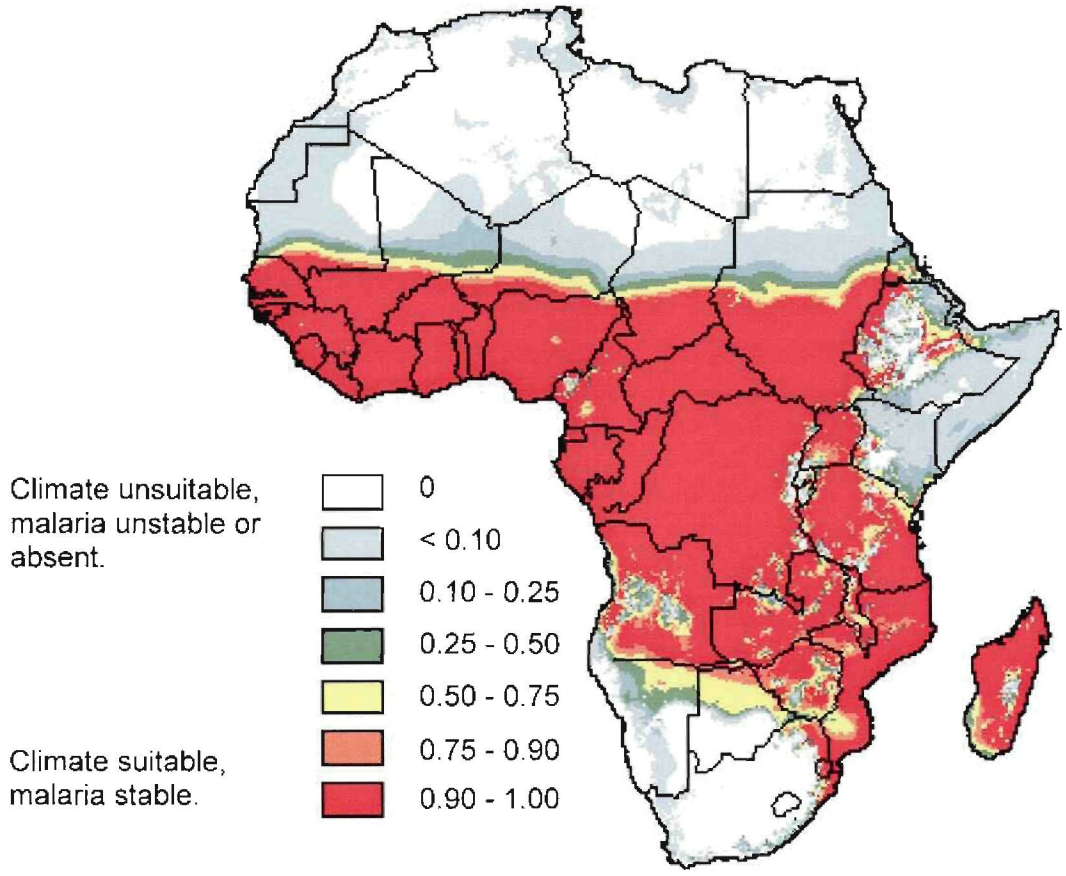

Figure 2.1: Continental distribution of malaria (MARA/ARMA, 2004).

The great impact of these diseases and the globally inadequate responses has counteracted health gains produced over the last ten years and contributes towards poverty considerably in numerous low- and middle-income countries (Theobald *et al.*, 2006). The WHO states that 90% of fatalities worldwide are in Africa, the majority once again being children under the age of 5 years. This fact is emphasized when examining the climate suitability for the disribution of malaria across Africa as illustrated in Figure 2.1. The annual malaria mortality rate in Senegal has increased considerably in children since the early 1990s (Cissé *et al.*, 2006).

Figure 2.2: Charles Louis Alphonse Laveran (Wikipedia, 2007)

Scientific studies on this parasitic infection made their first major advance in 1880. Charles Louis Alphonse Laveran (Figure 2.2), a doctor in the French armed forces working in Algeria, observed the parasites in red blood cells obtained from malaria patients, claiming that this protozoan causes malaria, making it the first time for protozoa to be identified as the cause of disease. The two Italian scientists Angelo Celli and Ettore Marchiafava named the protozoan *Plasmodium*. A year later, the Cuban doctor Carlos Finlay who was treating patients in Havana for yellow fever, was the first to suggest that disease was transmitted to humans by mosquitoes. But it was Sir Ronald Ross from Britain, working in India during that time, who in 1898, proved that mosquitoes transmitted malaria. He showed that malaria was transmitted to birds via certain species of mosquitoes and the malaria parasites were isolated from mosquitoes' salivary glands that fed on birds infected with the parasites (Wikipedia, 2007).

2.1.2 Lifecycle of *Plasmodium falciparum*

In the human body, malaria develops in two stages: an erythrocytic phase and a hepatic (exo-erythrocytic) phase. When a person's skin is pierced by a mosquito that has been infected with the parasite, sporozoites, present in the saliva of the mosquito enter the systemic circulation

and travel to the liver. The hepatocytes are infected by the parasite within 30 minutes after introduction to the human host where it will multiply asymptomatically and asexually for approximately 6 – 15 days. Often being referred to as hypnozoites, the sporozoites reside dormant within the liver at this time, where differentiation will take place to yield merozoites by thousands as illustrated in Figure 2.3. After the merozoites have ruptured their host cells, they will escape into the systemic circulation, infecting red blood cells and as a result begin the erythrocytic stage (Bledsoe, 2005). In order to escape the liver undetected, the parasite has to wrap itself in the membrane of the host liver cell (Strum *et al.*, 2006).

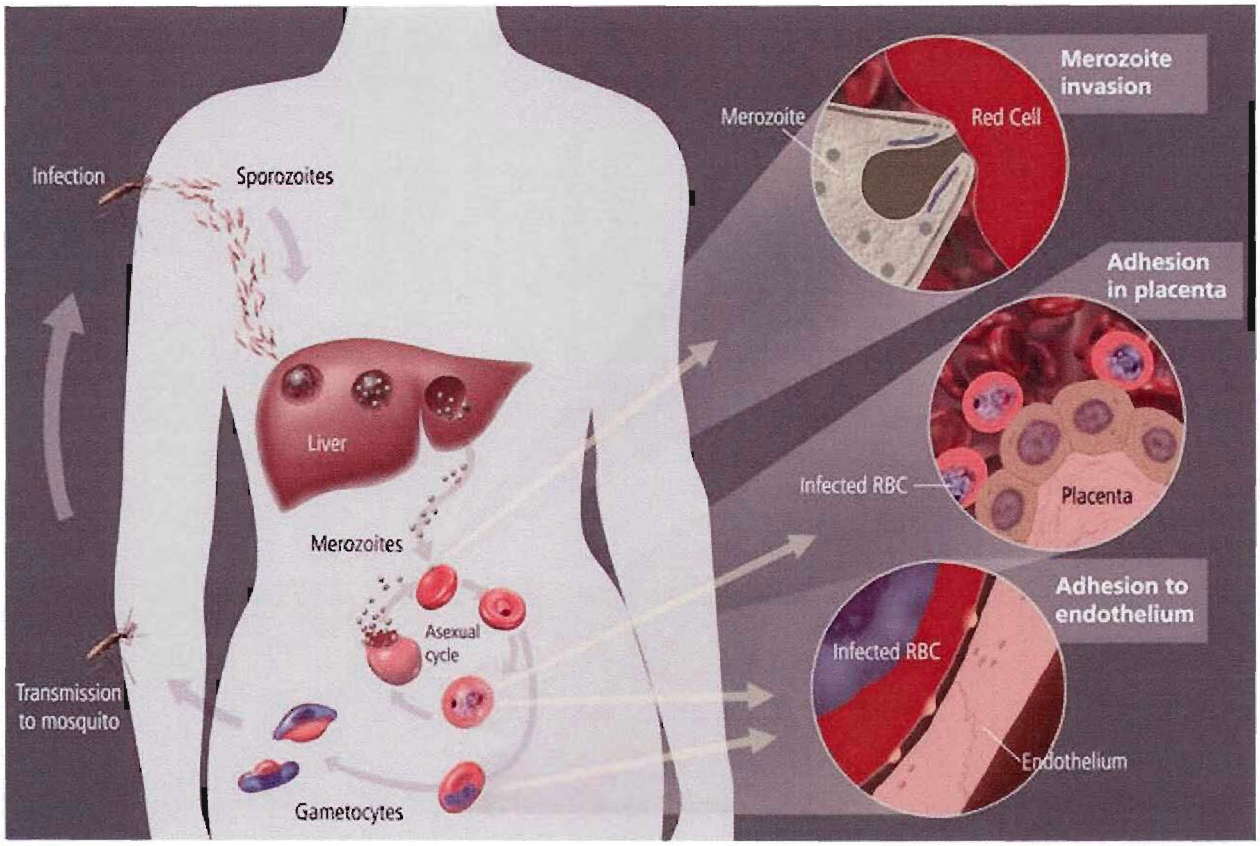

Figure 2.3: The lifecycle of *Plasmodium falciparum* (Wikipedia, 2007)

The parasites reproduce, once again asexually, inside these red blood cells and sporadically break out to invade fresh cells. These amplification cycles occur numerous times. Therefore, typical descriptions of fever waves occur from instantaneous waves of escaping merozoites that infect blood cells. Various *Plasmodium ovale* and *Plasmodium vivax* sporozoites do not instantly produce exo-erythrocytic-phase merozoites, but as a substitute produce hypnozoites that stay dormant for episodes ranging from 6 – 12 months to three years. Reactivation and production of merozoites follows after an episode of dormancy. Hypnozoites are accountable for late relapses and long incubation in the above mentioned two malaria species (Cogswell, 1992).

2.1.3 Symptoms and Diagnosis of Malaria

The clinical features of malaria include severe headaches, shivering attacks, sweat, joint and muscular pains, a fever above 38 °C and nausea. Cyanosis, fatigue, severe diarrhoea and respiration difficulty are some of the symptoms present in severe cases and may progress to complicated malaria once sleepiness, coma, unconsciousness, shock or convulsions occur (South Africa, 1998).

When diagnosing malaria, it is important to remember that the vital element is a suspicion of high index in endemic as well as non-endemic areas. Any person returning from or residing in a malaria area, who presents with flu-like symptoms and fever, should be tested (South Africa, 1998).

2.1.4 Treatment

Numerous classes of antimalarials are available and these drugs are categorized according to the selective actions they have on different stages of *plasmodium*'s life cycle. The tissue schizonticides are drugs with the action of eliminating dormant or developing liver forms; while blood schizonticides are those acting on the erythrocytic parasites; and the last group, called the gametocides are responsible for preventing transmission of the parasite to mosquitoes as well as exterminating the sexual stages (Rosenthal & Goldsmith, 2001).

The foundation in the prophylaxis of malaria is preventing mosquito bites. Even during the use of chemoprophylactic agents, non-medication measures must be strictly applied. These measures include the following:

- Endemic areas should be visited in the dry season.
- Wear ankle protectors, long trousers, long sleeves and light-coloured clothing when outdoors between dusk and dawn.
- Insect repellents that contain diethyltoluamide should be applied to clothing and skin that's exposed.
- Coils, screens and mosquito nets can also be used.
- Impregnate clothing and nets with the insecticide known as Peripel® (contains pyrethroid) (Gibbon, 1997).

Any person who is at risk of having a severe malaria attack should not enter a malaria area and must be discouraged from doing so. High risk groups include the following:

- Children under the age of 5.
- Pregnant women
- Elderly and immunity impaired patients.
- AIDS patients and immunocompromised patients on chemotherapy or long-term steroid therapy (Gibbon, 1997).

2.2 Sulfadoxine-pyrimethamine Combination Therapy

2.2.1 Introduction

Numerous studies indicated that children in Africa can be protected against malaria's consequences successfully by means of chemoprophylaxis, using antimalarials frequently, occasionally in doses lower than that of the therapeutic range (McGregor *et al.,* 1956; Greenwood *et al.,* 1988; Allen *et al.,* 1990; Menendez *et al.,* 1997; Geerligs *et al.,* 2003). Generally, the mortality in Gambian children decreased by approximately 35%, following treatment with pyrimethamine and dapsone taken in combination fortnightly during the transmission season of malaria (Greenwood *et al.,* 1988).

In pregnant women, preventive treatment, as the above mentioned, initially proved to be a successful approach in the management of malaria. Placental malaria was reduced by 72% in Malawi when the sulfadoxine-pyrimethamine combination was administered (Schultz *et al.,* 1994).

In some regions of Tanzania, where the transmission of the disease is perennial, a related approach was modified in two studies to prevent malaria in infants (Schellenberg *et al.,* 2001; Massaga *et al.,* 2003). Clinical episodes of anaemia and malaria, and the frequency thereof, were reduced by approximately two thirds (Verhoef *et al.,* 2002; Desai *et al.,* 2003).

2.2.2 Sulfadoxine and its pharmacological classification

Sulfadoxine is a long-acting sulfonamide with a half-life of 7 – 9 days and acts as an antifolate agent. It is absorbed well after oral intake and the urinary excretion thereof is extremely slow and in serum, this results in drug levels that are prolonged. The slow excretion of sulfadoxine is partially due to extensive tubular reabsorption and in part due to a protein binding exceeding

85%. Available as Fansidar®, sulfadoxine is used in combination with pyrimethamine as second-line treatment of malaria (Chambers, 2001).

2.2.3 The mechanism of action of sulfonamides

As competitive antagonists and structural analogs of PABA (para-aminobenzoic acid), sulfonamides act in the synthesis of pteroylglutamic acid (folic acid) by preventing PABA's normal bacterial utilization. More particularly, sulfonamides act as competitive inhibitors of the bacterial enzyme dihydropteroate synthase, liable for PABA's incorporation into dihydropteroic acid (folic acid's immediate precursor). Sensitive micro organisms have to produce their own folic acid, while unaffected bacteria are those that use preformed folate. Sulfonamide induced bacteriostasis is competitively counteracted by PABA. Since mammalian cells cannot produce their own folic acid, they are not affected by the mechanism of sulfonamides and can therefore be compared to sulfonamide-insensitive bacteria, which make use of preformed folate (Mandell & Petri, 1996).

2.2.4 Physico-chemical properties of sulfadoxine

Sulfadoxine is an odourless, white or creamy-white, crystalline powder. Other names for sulfadoxine include sulformethoxine, sulphormethoxine, sulphadoxine, sulphorthodimethoxine, sulforthomidine and sulfadimoxinum. Sulfadoxine is chemically known as N^1 – (5,6-dimethoxy-4-pyrimidinyl)sulfanilamide (Kapoor, 1988).

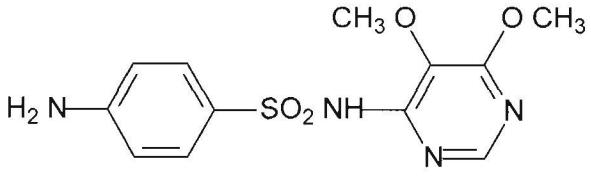

Figure 2.4: The chemical structure of sulfadoxine (Kapoor, 1988).

Sulfadoxine is slightly soluble in methanol and alcohol and its solubility in water is very slight. It is basically insoluble in ether but soluble in alkali solutions, i.e. carbonates and hydroxides, as well as diluted mineral acids. Suggested solvents for sulfonamides include both mono- and di-lower alkyl glycerol ethers. Sulfadoxine has an acidic nature and it melts between 197° and 200° (Kapoor, 1988).

2.2.5 Pyrimethamine and its pharmacological classification

Categorized as a blood schizontocide the slow-acting antimalarial, pyrimethamine, has equivalent *in vivo* effects to that of chloroguanide. Pyrimethamine's antimalarial potency is

greater though because it targets malarial parasites directly, and apart from that chloroguanide's active metabolite has a much shorter half-life than pyrimethamine. Pyrimethamine is of a pharmacological class called the diaminopyrimidines (Tracy & Webster, 1996).

2.2.6 The mechanism of action of Diaminopyrimidines

In a series of investigations, it was shown that the 2,4-diaminopyrimidines inhibit plasmodia's dihydrofolate reductase at much lower concentrations than required for similar inhibition of mammalian enzymes. The difference has since been shown between plasmodial dihydrofolate reductase and its mammalian counterparts in that the latter do not possess both thymidylate synthetase and dihydrofolate reductase activities. Two steps are inhibited in a vital metabolic pathway, and this inhibition explains pyrimethamine's synergism with sulfones and sulfonamides. The first step is the utilization of PABA in dihydropteroic acid's synthesis (inhibited by sulfonamides), while the second step consists of dihydrofolate's reduction to tetrahydrofolate (inhibited by pyrimethamine). Antifolates inhibit nuclear division by failure during the formation of schizonts in the liver and erythrocytes. This occurs late in the malarial parasite's life cycle. Compared to quinoline antimalarials, the antifolates have a slow onset, causing a consistent mechanism (Tracy & Webster, 1996).

2.2.7 Physico-chemical properties of pyrimethamine

Pyrimethamine is a white, tasteless, odourless, crystalline powder. The chemical name for pyrimethamine is 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine and in combination with sulfadoxine, it is known as Fansidar® (Loutfy & Aboul-Enein, 1983).

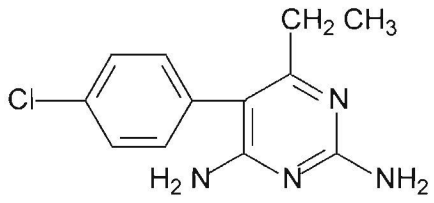

Figure 2.5: The chemical structure of pyrimethamine (Loutfy & Aboul-Enein, 1983).

In water, pyrimethamine is basically insoluble, however in ethanol, chloroform, acetone and dilute hydrochloric acid (HCl) it is slightly soluble (Loutfy & Aboul-Enein, 1983).

2.2.8 Clinical uses and adverse effects of the combination

The sulfadoxine-pyrimethamine combination is used in the treatment of uncomplicated *P. falciparum* malaria resistant to chloroquine. In adults, a single oral dose of 50/1000 mg to

75/1500 mg pyrimethamine/sulfadoxine should be taken (that is 2 – 3 Fansidar® tablets). The paediatric dose for children under the age of four is half a tablet; for 4 – 8 years, a tablet and for children 9 – 14 it is two tablets also as a single oral dose (Gibbon, 1997).

The contraindications for this combination include the following:

- Sulphonamide hypersensitivity.
- Folate deficiency.
- Megaloblastic anaemia.
- G6PD deficiency.
- Blood dyscrasias.
- Severe hepatic or renal impairment.
- Convulsive disorders (Gibbon, 1997).

Fansidar® is also contraindicated in neonates; pregnant women, as it crosses the placenta; lactating women, because of its excretion in breast milk and in patients with porphyria (Gibbon, 1997).

Drug interactions with Fansidar® include:

- Increased anti-folate effects with folate antagonists.
- Increased phenytoin levels with phenytoin.
- Enhanced hypoglycaemic effects with sulphonylureas.
- High risk of fatal skin reactions, predominantly in HIV patients, with chloroquine.
- Potentiated anticoagulant effects with warfarin (Gibbon, 1997).

Frequent adverse effects for the combination sulfadoxine-pyrimethamine are abdominal discomfort, nausea and vomiting, dizziness, headaches, skin reactions and photosensitivity. Leukopenia, megaloblastic anaemia, thrombocytopenia and hepatitis are rare adverse effects while more severe effects include toxic epidermal necrolysis, fatal skin reactions and Stevens-Johnson syndrome (Gibbon, 1997).

CHAPTER 3

METHODS AND EXPERIMENTS

3.1 Introduction

In the pharmaceutical industry it is vital that the available products meet the requirements stipulated by internationally recognised pharmacopoeias and their monographs. To ensure quality, safety and efficacy, these drugs need to withstand and pass several tests and procedures. One of the tests employed to determine efficacy is the dissolution test.

The definition of dissolution as stated by Aulton (Aulton, 2002) is the process that may be considered to involve the relocation of a solute molecule from an environment where it is surrounded by other identical molecules, with which it forms intermolecular attractions, into a cavity in a liquid, where it is surrounded by non-identical molecules, with which it may interact to different degrees (Aulton, 2002). The dissolution test is defined by the Pharmaceutical Codex as the test that shows the rate at which the drug passes into solution from the tablet and this is an important factor in controlling the availability of the drug (Pharmaceutical Codex, 1979).

A dissolution test requires a medium, apparatus and method (test conditions) that is reproducible and sufficiently rugged though discriminating. In general the dissolution test submits data to a decision of acceptance or rejection by means of the acceptance criteria. The criteria must be representative of various batches with similar manufacturing processes and nominal compositions, not excluding key batches used during fundamental studies, and in stability studies it should be representative of performance (USP, 2007).

Furthermore, the dissolution procedure should consist of the capability to distinguish substantial changes in a manufacturing process or composition that might affect the *in vivo* performance. There is a possibility that differences between batches might be illustrated by this procedure whilst *in vivo*, an insignificant difference is detected. Careful evaluation is required in this situation to determine if the procedure is appropriately discriminating or too sensitive. The assessment of results obtained from several batches representing typical variability in manufacturing parameters and composition may aid in this evaluation (USP, 2007).

Regarding stability, the dissolution procedure should appropriately reflect significant changes within the drug over time, caused by humidity, photosensitivity, temperature, and other possible influencing factors (USP, 2007).

When a test is well designed, the result should not be highly variable data, nor should it be associated with major analytical problems regarding the stability of the solution. Identifying trends or the effects of changes in the formulation is complicated when the results illustrate high variability (USP, 2007).

During a literature study various publications were found indicating that sulfadoxine/pyrimethamine products fail the aforementioned requirements frequently. In a study done by Kayumba (Kayumba *et al.*, 2004), several batches of drug samples were purchased in Rwanda, two of which contained sulfadoxine and pyrimethamine in combination. The *in vitro* dissolution and potency of the formulations were immediately evaluated after purchase and throughout the storage period of 6 months that occurred under a simulated tropical environment. The drugs were assayed and dissolution characteristics determined for each formulation by means of the USP 24 methods. The dissolution tests were carried out directly after purchase, then after storage of 3 months and lastly after 6 months (Kayumba *et al.*, 2004).

The content of both pyrimethamine and sulfadoxine were within the requirements of the USP 24 (90 – 110% labelled amount of pyrimethamine and sulfadoxine) and storage conditions did not affect the results. All the formulations met the requirements for dissolution testing stipulated in the USP 24 for sulfadoxine prior to stability testing, however it was detected that the release of sulfadoxine progressively decreased in the formulation obtained from Company A after 3 months of storage (67.6%) and a mere 44.4% was released after 6 months of storage. The tablets obtained from Company A failed the requirements for the release of pyrimethamine at time of purchase. The samples obtained from Company B showed a decrease in the release of pyrimethamine during the storage period; however, the limits of tolerance were not exceeded (Kayumba *et al.*, 2004).

In another study dissolution tests were performed on each of 18 different sulfadoxine/pyrimethamine brands utilizing the USP method. According to the requirements for dissolution testing, not less than 60% of pyrimethamine and sulfadoxine must dissolve within 30 minutes in the dissolution medium. Fansidar® (Roche) proved outstanding dissolution conformity and was used as a comparator. However, a total of eight samples (44.4%) failed to comply with the USP requirements. It wasn't specified if both or only one of the two active ingredients failed but it was stated that a low dissolution rate for pyrimethamine in numerous of the samples tested would produce a serious clinical impact (Minzi *et al.*, 2003).

An anti-malarial project was performed by Botwe (Botwe *et al.*, 2005) with one of the objectives being the validation of analytical methods. These methods were developed between the USP / DQI (United States Pharmacopoeia / Drug Quality and Information Program) and the FDB (Food and Drug Board - Ghana). An HPLC system was fitted with a 250 x 4.6 mm Princeton

Sphere (C-18) column, particle size 5 µm. The mobile phase used was a mixture of acetonitrile (0.1 M) and phosphate buffer (pH 4) to the ratio 70 : 30. A flow rate of 2 ml per minute was maintained whilst the injection volume was 10 µl. For the testing of dissolution characteristics of pyrimethamine and sulfadoxine tablets, according to the dissolution monograph, the USP 24 / F.D.B. apparatus and a modified method were used. The parameters for this dissolution test were:

Apparatus:	2 (Paddles)
Time:	30 min
Dissolution medium:	Phosphate buffer, pH 6.8 (900 ml)
Speed:	75 rpm (Botwe *et al.*, 2005).

Six tablets were weighed and then introduced into six dissolution vessels. After 30 minutes, a filtered portion (20 ml) of dissolution solution was sampled into a volumetric flask (50 ml). To this solution, 9 ml of pyrimethamine (USP RS) standard solution (0.5 mg/ml) was added and diluted to volume. Analysis of the solution was performed according to the modified assay method of sulfadoxine and pyrimethamine tablets. Of the 25 tested samples, four failed the dissolution requirements with regards to sulfadoxine while all the samples passed dissolution for pyrimethamine (Botwe *et al.*, 2005).

3.2 Instruments and Apparatus

All the experiments in this study were performed in a SANAS (South African National Accreditation System) accredited laboratory.

3.2.1 The HPLC system

The HPLC system, manufactured by THERMO SEPARATIONS®, consisted of a spectraSYSTEM P1000 isocratic pump, a spectraSYSTEM AS 3000 autosampler with a variable volume loop injector and a spectraSYSTEM UV 1000 programmable variable wavelength detector with a 10 mm analytical flow cell. A spectraSYSTEM SN 4000 signal converter was used to convert the analog detector signal to a digital signal. The converted signal was fed into a Pentium® II computer with a Windows NT® Workstation version 4.00 operating system, where the signal was integrated by means of Chromquest® version 2.53 software. The HPLC was fitted with a 250 x 4.6 mm Phenomenex column (Luna C-18), particle size 5 µm. The UV wavelength used for identification of the two active ingredients was 254 nm, the flow rate 2 ml per minute and the injection volume 10 µl.

3.2.2 Dissolution Apparatus

For this study, two dissolution apparatus were used, the first was an Erweka® (DT6R) dissolution apparatus, fitted with a thermostat, regulating the temperature of the dissolution medium at 37 ± 0.5 °C, and synchronous motor (Erweka®, Heustenstamm, Germany) with adjustable speed settings. The second dissolution apparatus used was a Van Kel® (VK 7000), also fitted with a thermostat which regulated the temperature of the dissolution medium at 37 ± 0.5 °C, and synchronous motor (Van Kel®, Edison, NJ, U.S.A.). Both the apparatus used for dissolution testing consisted of seven vessels, six of which were used for the dissolution test of six sample tablets at a time while the seventh held dissolution medium for replacement after each sampling. The volume of dissolution medium used in each vessel was a 1000 ml and the apparatus were fitted with paddles which rotated at 75 rpm.

3.3 Method Development

Method development is important in this study as it promotes a changed HPLC analytical method for determining the active ingredients after dissolution testing was performed. Certain analytical parameters that may influence the dissolution test results negatively were changed. The method suggested by this study is thus a modified depiction of the existing USP method.

3.3.1 USP Method

The USP states that, for the dissolution test of the combination sulfadoxine and pyrimethamine tablets, a 1000 ml of phosphate buffer solution (PBS) with a pH of 6.8 is used as dissolution medium, using Apparatus 2 (paddles) that rotates at 75 rpm for 30 minutes (USP, 2006).

This procedure is followed in order to determine the amounts of pyrimethamine and sulfadoxine dissolved, using the procedure described in the *Assay* and if necessary modifications are to be made. It is stated in the tolerances that no less than 60% of each of pyrimethamine and sulfadoxine's labelled amount should be dissolved within 30 minutes (USP, 2006).

Mobile phase:
Prepare a suitable filtered and degassed mixture of dilute acetic acid glacial (1%) and acetonitrile (4 : 1) (USP, 2006).

Internal standard solution:
Prepare a phenacetin in acetonitrile solution with a concentration of 1 mg/ml (USP, 2006).

Standard stock solution:

Accurately weigh approximately 25 mg USP pyrimethamine RS and 500 mg USP sulfadoxine RS, transfer to a volumetric flask (100 ml) and dissolve in 35 ml acetonitrile. Finally dilute the solution to volume with *mobile phase*, and mix (USP, 2006).

Standard preparation 1:

Pipet 2 ml *internal standard solution* and 25 ml *standard stock solution* into a volumetric flask (50 ml), dilute to volume with *mobile phase*, and mix (USP, 2006).

Standard solution 2:

Pipet 10 ml *internal standard solution* and 2 ml *standard stock solution* into a volumetric flask (250 ml), dilute to volume with *mobile phase*, and mix (USP, 2006).

3.3.2 Modified Method A

During this study some modifications were made. Firstly, the *internal standard solution* was not used and secondly, the standard solution as specified was altered. Secondary standards (verified against USP primary standards) were used and two standard stock solutions were prepared, the first of which contained approximately 500 mg of sulfadoxine in a 100 ml of acetonitrile, and the second contained about 25 mg of pyrimethamine in a 100 ml of acetonitrile. From these two stock solutions, three sets of dilutions were made as follow:

1. 1 ml Sulfadoxine solution → 10 ml
2. 1 ml Pyrimethamine solution → 10 ml
3. 1 ml Sulfadoxine and 1 ml pyrimethamine solution → 10 ml

For each batch (consisting of three sets of dilutions as shown above) a different solvent was used, i.e. 0.1 N HCl, PBS pH 6.8 and mobile phase respectively. These dilutions were used as standards and a duplicate batch was considered the samples. The mobile phase, as specified by the USP, was used in this method. The samples together with the standards were analysed by means of HPLC and the results obtained.

3.3.3 Modified Method B

In this method the mobile phase, as specified by the USP, was modified by adjusting the pH to 4 with sodium hydroxide (10 M) in order to produce a more alkaline mobile phase. The two standard stock solutions, one containing about 500 mg sulfadoxine per 100 ml acetonitrile and the other containing approximately 25 mg pyrimethamine per 100 ml acetonitrile, were prepared and the dilutions were made as described in Method A.

These dilutions were used as standards and a duplicate batch was considered the samples. The samples together with the standards were analysed by means of HPLC and the results obtained. Upon evaluation of the data, Method B resulted in the method of choice.

3.4 Validation

For validation, the mobile phase as prescribed in Method B was used. The following parameters were evaluated: linearity, range, repeatability and accuracy. The definitions and limits of these parameters as stated by the International Conference on Harmonisation (I.C.H.) are given below.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. The regression line's y-intercept, correlation coefficient and slope, together with the residual sum of squares must be submitted, including a plot of the data. For the purpose of this study a correlation coefficient larger then 0.98 must be obtained. The deviation of the regression line's data points could be analysed to assist in evaluating linearity. It is recommended to use no less then five concentrations to establish linearity (ICH, 1994; ICH, 1996).

Range:

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The limit for dissolution testing is +/- 20% over the specified range (ICH, 1994; ICH, 1996).

Repeatability:

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (e.g. 3 concentrations/3 replicates each) (ICH, 1994; ICH, 1996).

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Numerous methods exist for determining accuracy, for the purpose of this study accuracy was inferred after establishment of linearity, precision and specificity (ICH, 1994; ICH, 1996).

Four standard stock solutions were prepared, two of which contained approximately 50 mg sulfadoxine, weighed accurately, in 50 ml acetonitrile, whilst the other two contained approximately 50 mg pyrimethamine, accurately weighed, in 200 ml acetonitrile. The two sulfadoxine standard stock solutions were marked S1 and S2 respectively, while the two pyrimethamine standard stock solutions were labelled P1 and P2 respectively for identification as shown below.

Sulfadoxine standard stock solutions:

S1: 50.15 mg → 50 ml acetonitrile

S2: 50.22 mg → 50 ml acetonitrile

Pyrimethamine standard stock solutions:

P1: 50.07 mg → 200 ml acetonitrile

P2: 50.25 mg → 200 ml acetonitrile

These stock solutions were used for the validation of Method B using 0.1 N HCl, PBS pH 6.8 and water in the dilutions. Thus three batches of dilutions were prepared, the first batch where 0.1 N HCl was used as solvent, the second where PBS (pH 6.8) was used as solvent and the third where water was the solvent. The following dilutions were prepared:

Validation standards:

1. 15 ml S1 + 3 ml P1 → 20 ml
2. 10 ml S1 + 2 ml P1 → 15 ml
3. 5 ml S1 + 1 ml P1 → 10 ml
4. 5 ml S1 + 1 ml P1 → 15 ml
5. 5 ml S1 + 1 ml P1 → 20 ml

Control standard:

5 ml S2 + 1 ml P2 → 10 ml

After the preparation of these dilutions, the standards were analysed by means of HPLC. All the *validation standards*, except for *validation standard 3*, were injected three times on the HPLC to establish repeatability whilst *validation standard 3* was injected five times to ensure system suitability. To determine accuracy, the *control standard* was injected twice. The five *validation standards* covered the range of 50% to 150% to establish linearity.

3.5 Dissolution testing of Fansidar®

The dissolution tests of Fansidar® were performed in three different mediums (0.1 N HCl, PBS pH 6.8 and water) under the following specifications:

Apparatus type:	2 (Paddles)
Speed:	75 rpm
Temperature:	37 °C
Duration:	60 min
Q-time:	30 min
Q-value:	60 %

With each dissolution test, six tablets were individually weighed and introduced into the dissolution vessels. Each vessel contained 1000 ml dissolution medium of which the temperature was approximately 37 °C. Samples of 5 ml were withdrawn from each vessel through a filter (0.45 µm pore size) at the following sample times: 10 minutes, 20 minutes, 30 minutes, 45 minutes and 60 minutes. After taking a sample, the amount withdrawn was replaced with dissolution medium (5 ml). No dilutions were made and the samples together with their standards (discussed below) were analysed by means of HPLC, using the mobile phase described in Method B.

3.5.1 Fansidar® in 0.1 N HCl:

0.1 N HCl was prepared, filtered and degassed, after which each of the six dissolution vessels was filled with 1000 ml of the medium. The standard stock solutions and dilutions were prepared as follows:

Standard stock solutions:

Sulfadoxine:

S1: 50.7 mg → 50 ml acetonitrile

S2: 50.7 mg → 50 ml acetonitrile

Pyrimethamine:

P1: 50.9 mg → 200 ml acetonitrile

P2: 50.7 mg → 200 ml acetonitrile

Dilutions:

Standard 1: 5 ml S1 + 1 ml P1 → 10 ml 0.1 N HCl

Standard 2: 5 ml S2 + 1 ml P2 → 10 ml 0.1 N HCl

3.5.2 Fansidar® in pH 6.8 PBS:

For this dissolution test a PBS was prepared, the pH adjusted to 6.8, the mixture was filtered and degassed, and finally each of the six dissolution vessels was filled with 1000 ml of the medium. The standard stock solutions and dilutions were prepared as follows:

Standard stock solutions:

Sulfadoxine:

S1: 50.7 mg → 50 ml acetonitrile

S2: 50.7 mg → 50 ml acetonitrile

Pyrimethamine:

P1: 50.9 mg → 200 ml acetonitrile

P2: 50.7 mg → 200 ml acetonitrile

Dilutions:

Standard 1: 5 ml S1 + 1 ml P1 → 10 ml PBS (pH 6.8)

Standard 2: 5 ml S2 + 1 ml P2 → 10 ml PBS (pH 6.8)

3.5.3 Fansidar® in water:

Water was filtered and degassed for the dissolution test of Fansidar® in water as dissolution medium. The six vessels were individually filled with a 1000 ml of water and the standard stock solutions together with the dilutions were prepared as indicated below.

Standard stock solutions:

Sulfadoxine:

S1: 50.4 mg → 50 ml acetonitrile

S2: 49.6 mg → 50 ml acetonitrile

Pyrimethamine:

P1: 50.0 mg → 200 ml acetonitrile

P2: 50.2 mg → 200 ml acetonitrile

Dilutions:

Standard 1: 5 ml S1 + 1 ml P1 → 10 ml H_2O

Standard 2: 5 ml S2 + 1 ml P2 → 10 ml H_2O

Note that the validation of Fansidar® in water was performed and analysed on the HPLC together with the samples from the dissolution test. *Standard 1* and *standard 2* were used as validation standard 3 and the control standard respectively for system suitability during the analysis of the dissolution test samples.

3.6 Dissolution testing of Falcistat®

Falcistat® is a generic sulfadoxine/pyrimethamine product available on the Namibian market. Since no generic product is available on the South African market, this product was used as the reference generic product. The three dissolution tests as described for Fansidar® were repeated for Falcistat®, using the same specifications, mobile phase and dissolution mediums. The reason for this was to compare the results obtained from the Falcistat® dissolution tests, to that of the innovator product. The standard stock solutions and dilutions were also prepared as described for the dissolution testing of Fansidar®.

After completion of the dissolution tests and preparation of the standards, the samples together with their standards were analysed on the HPLC and the results obtained, processed.

3.7 Dissolution tests performed to indicate stability

After both the innovator product and one of its generics were tested in three different dissolution mediums, it was decided that the dissolution medium of choice is 0.1 N HCl, as it produced the best discriminatory results for both Fansidar® and Falcistat®. However, the stability of the two active ingredients (sulfadoxine and pyrimethamine) in 0.1 N HCl over time was questioned and dissolution tests in both 0.1 N and 0.01 N HCl, was suggested.

One tablet of each was tested in both 0.1 N and 0.01 N HCl as follow:

Vessel 1: One Fansidar® tablet in 0.01 N HCl (1000 ml)

Vessel 2: One Fansidar® tablet in 0.1 N HCl (1000 ml)

Vessel 3: One Falcistat® tablet in 0.01 N HCl (1000 ml)

Vessel 4: One Falcistat® tablet in 0.1 N HCl (1000 ml)

A 30 minute dissolution test was performed on all four tablets and one single 5 ml sample was taken from each vessel. The mobile phase as prescribed in Method B was used and the standard stock solutions together with their dilutions (using 0.01 N HCl as solvent) were prepared and analysed as prescribed for the dissolution testing of Fansidar® with HCl as dissolution medium.

The samples together with their standards were analysed over a 24 hour period on the HPLC in order to confirm which one of the two mediums proved better stability. From results it was obvious that 0.1 N HCl still remained the dissolution medium of choice.

3.8 Dissolution testing of generic products

Dissolution tests were performed on seven other generic products in order to establish whether the outcome would remain the same as for the innovator product. Seven generic products were obtained from Tanzania, i.e. Laridox®, Orodar®, Tansidar®, Sulphadar®, Malostat®, Dionsdar® and Sulfadoxine & Pyrimethamine Tablets USP. Dissolution tests, utilizing 0.1 N HCl as dissolution medium, were performed for all these products and the standard stock solutions and dilutions were prepared as described for the dissolution testing of Fansidar® using the dissolution medium as solvent for the dilutions.

All the samples and their standards were analysed on the HPLC, using the mobile phase from Method B, and the results obtained.

Eventhough sulfadoxine in Dionsdar® failed to comply with the requirements, it was Tansidar®'s dissolution test that was repeated, using PBS (pH 6.8) as dissolution medium (the medium specified by the USP) to establish whether better results would be obtained. The reason for this was that both sulfadoxine and pyrimethamine failed to comply with the requirements stated in the USP and the concern here was the dissolution properties of pyrimethamine.

4.1 Introduction

All the results obtained after analysis on the HPLC were processed and the data submitted to the tolerances and specifications as stipulated in the USP. These results are now presented and discussed in this chapter and all graphs and figures referred to are included in appendix 1.

4.2 Method Development

As specified in chapter 3, the method development was performed first to establish the method of choice for this study. The analytical value referred to in each table is the area under the curve (AUC) obtained from each chromatogram. For each set of results the average, standard deviation (SD) and relative standard deviation (% RSD) were calculated. For the purpose of system suitability and repeatability of an HPLC analysis, the USP specifies that the % RSD for five or six standard determinations (depending on the specific monograph) should be less than or equal to 2% (USP, 2007).

4.2.1 Method A

The following results were obtained for the analysis of the samples tested for Method A.

Table 4.1: Comparative peak areas (analytical value) for sulfadoxine as a single component in standard solutions (Method A).

	Sulfadoxine (single)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	13626840	13653096	13699553
	13548098	13537538	13710654
	13618822	13563745	13643010
	13495386	13511990	13546364
	13457149	13517545	13520871
	13450874	13515488	13470189
Average	13532861	13549900	13598440
SD	77861	54151	99979
% RSD	0.575	0.400	0.735

Table 4.2: Comparative peak areas (analytical value) for sulfadoxine in combination with pyrimethamine in standard solutions (Method A).

	Sulfadoxine (combination)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	13632373	13589746	13410183
	13762097	13741817	13385210
	13730809	13699897	13369281
	13644085	13522911	13493797
	13617588	13521570	13705061
	13633910	13655331	13608002
Average	13670144	13621879	13495256
SD	60525	92174	135609
% RSD	0.443	0.677	1.005

The % RSD for each batch of dilutions in Table 4.1 complies with the USP specifications for sulfadoxine single component. The same can be stated for the combination, however, the % RSD for the samples diluted with HCl (0.1 N) in the combination is the smallest, while for the single component the % RSD for the samples diluted with PBS is the smallest.

For pyrimethamine, the results shown below were obtained. The results for both the single component and the combination are given in two separate tables.

Table 4.3: Comparative peak areas (analytical value) for pyrimethamine as a single component in standard solutions (Method A).

	Pyrimethamine (single)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	512149	338299	521111
	512315	341740	507080
	512058	337574	507757
	517867	336515	506582
	518756	335820	510065
	520279	334915	508196
Average	515571	337477	510132
SD	3801	2412	5511
% RSD	0.737	0.715	1.080

Table 4.4: Comparative peak areas (analytical value) for pyrimethamine in combination with sulfadoxine in standard solutions (Method A).

	Pyrimethamine (combination)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	580828	482294	559829
	593248	491693	558802
	589645	483508	553764
	586899	479532	571565
	583445	477058	587867
	580980	484915	577788
Average	585841	483167	568269
SD	4999	5043	13091
% RSD	0.853	1.044	2.304

The % RSD for the PBS dilutions of the single component was the smallest while the % RSD obtained for the dilutions with 0.1 N HCl of the combination was smallest. The % RSD for all the sets of samples of both the single component pyrimethamine (Table 4.3) and the combination (Table 4.4) complied with the USP specifications except for the dilutions in mobile phase of the combination. The overall % RSD for sulfadoxine and pyrimethamine with Method A was 0.47 and 22.23, respectively.

The results clearly indicated that the AUC for pyrimethamine in PBS is significantly smaller than when other media was used for dilution. This may be attributed to either the properties of the solution or interferences from other peaks. Further investigation resulted in the conclusion that impurities originating from the sulfadoxine peak may have interfered with the detection of the pyrimethamine peak, thus an alteration had to be made in order to detect a true pyrimethamine peak value. Therefore, the pH of the mobile phase was altered to produce a more alkaline mobile phase and in doing so, forcing the pyrimethamine peak to appear after the sulfadoxine peak, preventing sulfadoxine impurities from interfering with the pyrimethamine peak and so, Method B was developed.

4.2.2 Method B

The results obtained after analysis on the HPLC for both sulfadoxine and pyrimethamine are presented in the four tables below. The mobile phase used had a pH of 4.

Table 4.5: Comparative peak areas (analytical value) for sulfadoxine as a single component in standard solutions (Method B).

	Sulfadoxine (single)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	13799052	13791972	13830900
	13800483	13803916	13814529
	13825544	13830860	13833631
	13848140	13480469	13736773
	13854951	13853112	13756310
	13851290	13822935	13741710
Average	13829910	13763877	13785642
SD	25510	140464	45529
% RSD	0.185	1.021	0.330

Table 4.6: Comparative peak areas (analytical value) for sulfadoxine in combination with pyrimethamine in standard solutions (Method B).

	Sulfadoxine (combination)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	13708759	13823968	13675592
	13883002	13895882	13791081
	13819272	13944745	13777322
	13970806	13892828	13854331
	13984904	13869986	13787933
	14011984	13860475	13820035
Average	13896455	13881314	13784382
SD	116676	40545	60177
% RSD	0.840	0.292	0.437

Sulfadoxine, the single component, diluted in 0.1 N HCl produced the best % RSD, but when the combination was analysed, sulfadoxine in PBS presented the smallest % RSD. All % RSDs for sulfadoxine complied with the requirements as specified by the USP.

Table 4.7: Comparative peak areas (analytical value) for pyrimethamine as a single component in standard solutions (Method B).

	Pyrimethamine (single)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	539248	377274	529454
	528114	376296	529350
	554835	373184	528682
	543321	354745	519281
	551275	355592	518879
	547749	354361	519338
Average	544090	365242	524164
SD	9589	11417	5484
% RSD	1.762	3.126	1.046

Table 4.8 Comparative peak areas (analytical value) for pyrimethamine in combination with sulfadoxine in standard solutions (Method B).

	Pyrimethamine (combination)		
	0.1 N HCl	***PBS (pH 6.8)***	***Mobile phase***
Analytical value	477462	431935	509076
	500859	434584	519095
	458442	435751	525380
	286785	448446	528566
	424818	447696	517026
	511875	448397	522343
Average	443374	441135	520248
SD	82765	7820	6877
% RSD	18.667	1.773	1.322

The single component of pyrimethamine produced a % RSD above 2 in PBS, as did the combination in HCl. However, both the single component and the combination diluted in mobile phase produced the best % RSDs.

Upon examining the results obtained for pyrimethamine (combination) in 0.1 N HCl it was noticed that analytical value 4 appeared to be an outlier and responsible for a very high % RSD. This could be due to air in the system or a power failure or surge. Taking this into account, the value was discarded and a recalculated % RSD of 7.318 obtained. Even though not all the % RSDs complied with USP requirements, the overall % RSD for sulfadoxine and pyrimethamine with Method B was 0.40 and 15.78, respectively. Hence, for pyrimethamine a

remarkably smaller % RSD was obtained with Method B and validation proceeded for this method.

4.3 Validation

Three validations of the combination sulfadoxine and pyrimethamine were performed to establish linearity, repeatability, range and recovery. For each validation a different diluent was used (PBS (pH 6.8), 0.1 N HCl and water). Method B was used for all validations.

4.3.1 PBS (pH 6.8)

Table 4.9: Validation results of sulfadoxine in PBS (pH 6.8).

Sulfadoxine (PBS pH 6.8)							
Conc.	***Area 1***	***Area 2***	***Area 3***	***Area 4***	***Area 5***	***Average***	***% RSD***
750.25	10355870	10399334	10360074			10371759	0.231
666.93	9133353	9125027	9134322			9130901	0.056
500.20	6832957	6908802	6935697	6910090	6901220	6897753	0.558
333.46	4584496	4578960	4583508			4582321	0.064
250.10	3420925	3429145	3416446			3422172	0.188

Name	***Area***	***Conc.***	***Av.***	***SD***	***% RSD***	***% Recovery***
Contr. 1-1	6810819	495.11	496.71	2.26	0.455	**99.16**
Contr. 1-2	6854964	498.31				

The correlation coefficient (r^2) for sulfadoxine was 0.9999 whilst the slope and y-intercept was 13817 and - 29902, respectively as illustrated in Figure A.1.

Table 4.10: Validation results of pyrimethamine in PBS (pH 6.8).

Pyrimethamine (PBS pH 6.8)							
Conc.	**Area 1**	**Area 2**	**Area 3**	**Area 4**	**Area 5**	**Average**	**% RSD**
36.69	365216	379142	345511			363290	4.651
32.61	322217	315219	369926			335787	8.866
24.46	253701	242687	229628	239588	261491	245419	5.064
16.31	168101	151653	167897			162550	5.806
12.23	107149	117713	118118			114327	5.440

Name	***Area***	***Conc.***	***Av.***	***SD***	***% RSD***	***% Recovery***
Contr. 1-1	261402	26.12	25.24	1.24	4.928	**102.81**
Contr. 1-2	243253	24.36				

For pyrimethamine the correlation coefficient was 0.9972; the slope 10317 and the y-intercept - 8070.7 as shown in Figure A.2. All sulfadoxine's % RSDs were lower than 2 whilst pyrimethamine produced % RSDs higher than 2.

Both sulfadoxine and pyrimethamine complied with the validation criteria with PBS (pH 6.8) as the diluent. The recovery for sulfadoxine and pyrimethamine was 99.16% and 102.81%, respectively.

4.3.2 HCl (0.1 N)

Table 4.11: Validation results of sulfadoxine in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)							
Conc.	**Area 1**	**Area 2**	**Area 3**	**Area 4**	**Area 5**	**Average**	**% RSD**
751.34	9958594	10068811	10134005			10053803	0.882
667.86	9152567	9180100	9116450			9149706	0.349
500.90	6814337	6811468	6818978	6822110	6794255	6812230	0.159
333.93	4513041	4511654	4542334			4522343	0.383
250.45	3400289	3399798	3378732			3392940	0.363

Name	***Area***	***Conc.***	***Av.***	***SD***	***% RSD***	***% Recovery***
Contr. 1-1	6782102	500.59	500.05	0.77	0.154	**99.97**
Contr. 1-2	6767434	499.50				

The correlation coefficient for sulfadoxine was 0.9992; the slope 13470 and the y-intercept 39070 as presented in Figure A.3.

Table 4.12: Validation results of pyrimethamine in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)							
Conc.	**Area 1**	**Area 2**	**Area 3**	**Area 4**	**Area 5**	**Average**	**% RSD**
36.82	345217	344318	360509			350015	2.600
32.73	339199	337052	337329			337860	0.346
24.55	253740	263097	263109	255104	256918	258394	1.720
16.37	168921	172517	169089			170176	1.193
12.27	122881	126946	125574			125134	1.653

Name	***Area***	***Conc.***	***Av.***	***SD***	***% RSD***	***% Recovery***
Contr. 1-1	254175	25.17	25.82	0.92	3.565	**105.54**
Contr. 1-2	266535	26.47				

For pyrimethamine, the correlation coefficient obtained was 0.9873; the slope was 9495.4 and the y-intercept was 15222 as shown in Figure A.4. The % RSDs obtained for sulfadoxine were all lower than 2. Pyrimethamine proved to have better repeatability with the 0.1 N HCl validation as lower % RSDs were obtained.

Sulfadoxine and pyrimethamine complied with the validation criteria with 0.1 N HCl as diluent and the recovery for sulfadoxine and pyrimethamine was 99.97% and 105.54%, respectively. When compared with the validation where PBS was used as diluent, both sulfadoxine and pyrimethamine produced higher recovery values with 0.1 N HCl.

4.3.3 Water

Table 4.13: Validation results of sulfadoxine in H_2O.

Sulfadoxine (H_2O)							
Conc.	**Area 1**	**Area 2**	**Area 3**	**Area 4**	**Area 5**	**Average**	**% RSD**
754.04	20756252	20651121	20655758			20687710	0.287
670.25	18295142	18251762	18270491			18272465	0.119
502.69	13875838	13715139	13763363	13739063	13796609	13778002	0.453
335.13	9150846	9172301	9187633			9170260	0.202
251.35	6957995	6964977	7021612			6981528	0.500

Name	***Area***	***Conc.***	***Av.***	***SD***	***% RSD***	***% Recovery***
Contr. 1-1	13586119	495.65	497.04	1.97	0.397	**100.47**
Contr. 1-2	13662067	498.44				

Table 4.14: Validation results of pyrimethamine in H_2O.

Pyrimethamine (H_2O)							
Conc.	**Area 1**	**Area 2**	**Area 3**	**Area 4**	**Area 5**	**Average**	**% RSD**
36.64	655067	1629741	2116529			1467112	50.724
32.57	1834217	604801	592246			1010421	70.610
24.43	461384	457337	446231	391944	368970	425173	9.876
16.28	294348	299045	295126			296173	0.850
12.21	208915	209079	204635			207543	1.214

Name	***Area***	***Conc.***	***Av.***	***SD***	***% RSD***	***% Recovery***
Contr. 1-1	460272	19.93	19.81	0.18	0.883	**80.79**
Contr. 1-2	448105	19.69				

The regression line for sulfadoxine presented a correlation coefficient of 0.9999, a slope of 27233 and a y-intercept of 87940 as illustrated in Figure A.5. For pyrimethamine the correlation coefficient was 0.8957, the slope 49182 and the y-intercept -520025 as shown in Figure A.6. The % RSDs obtained with sulfadoxine were lower than 2 while with pyrimethamine three % RSDs were obtained that were higher than 2 and three lower than 2. Due to these huge differences, poor repeatability was obtained, which resulted in a low % recovery.

Sulfadoxine complied with the validation criteria with a % recovery of 100.47% while pyrimethamine produced a % recovery of only 80.79%.

4.4 The dissolution testing of Fansidar®

The most important factor to consider when a dissolution test is performed is whether the product tested meets the requirements in terms of the Q-value (amount of product dissolved at time X) and the Q-time (the time in which a certain amount of product should be dissolved) as stated by the pharmacopoeia from which the method used originates. In this case no less than 60 (+5)% (Q-value +5%) of the labelled amount of both sulfadoxine and pyrimethamine should dissolve in 30 minutes (Q-time) for 6 individual test units (stage 1 criteria for dissolution). These specifications are applicable to the innovator product and all generic products tested in this study.

4.4.1 Fansidar® in pH 6.8 PBS:

Table 4.15: Percentage dissolution of sulfadoxine in Fansidar® in PBS (pH 6.8).

Sulfadoxine (PBS pH 6.8)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
10	54.17	62.71	67.61	66.33	67.12	68.28	64.37	8.338
20	80.34	86.39	82.83	83.93	82.10	84.77	83.39	2.546
30	89.14	92.45	88.99	89.99	87.98	89.89	89.74	1.686
45	93.42	94.49	91.25	92.52	91.55	92.69	92.65	1.290
60	95.01	94.99	92.49	93.27	93.62	94.61	94.00	1.100

Table 4.16: Percentage dissolution of pyrimethamine in Fansidar® in PBS (pH 6.8).

Pyrimethamine (PBS pH 6.8)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	18.69	11.60	19.88	24.72	24.68	26.40	21.00	26.227
20	30.05	32.18	31.49	33.00	34.23	59.04	36.66	30.150
30	33.97	36.39	34.84	36.15	36.12	36.61	35.68	2.912
45	38.14	39.65	24.78	36.76	38.76	32.25	35.06	16.175
60	40.62	40.75	38.68	38.85	40.92	41.56	40.23	2.933

Sulfadoxine complied with the dissolution test requirements as 89.7% dissolved in 30 minutes, and it is noteworthy that 64.4% had already dissolved within the first 10 minutes. Pyrimethamine however, failed to comply with the requirements with 35.7% dissolved in 30 minutes and at 60 minutes (twice the USP specified time) only 40.2% pyrimethamine was dissolved.

4.4.2 Fansidar® in 0.1 N HCl:

Fansidar® was also tested in 0.1 N HCl. The results are illustrated in the two tables below.

Table 4.17: Percentage dissolution of sulfadoxine in Fansidar® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	55.03	56.36	60.28	59.06	52.23	55.54	56.42	5.143
20	73.60	77.65	75.71	76.36	70.70	74.51	74.76	3.260
30	81.17	85.03	82.94	83.20	79.28	81.88	82.25	2.385
45	86.56	90.17	87.63	88.21	85.25	86.89	87.45	1.911
60	89.04	91.38	90.01	90.45	87.99	89.50	89.73	1.307

Sulfadoxine complied with the requirements, with 82.3% dissolved in 30 minutes. Pyrimethamine also complied with the requirements with 99.6% pyrimethamine dissolved in 30 minutes. This emphasizes the statement that PBS is not a suitable dissolution medium for this combination tablet. In fact, according to the USP 0.1 N HCl is used as dissolution medium for the single component pyrimethamine tablets (USP, 2006).

Table 4.18: Percentage dissolution of pyrimethamine in Fansidar® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	90.57	99.49	98.09	91.98	92.33	91.28	93.96	4.063
20	98.81	101.05	99.83	99.45	98.41	102.27	99.97	1.451
30	99.14	99.77	99.08	99.64	100.09	99.91	99.60	0.413
45	99.20	100.00	100.71	99.61	103.94	99.94	100.57	1.718
60	97.27	98.79	99.89	99.65	93.14	98.14	97.81	2.539

4.4.3 Fansidar® in water:

The results obtained for the dissolution of sulfadoxine and pyrimethamine in water, are illustrated in Table 4.19 and Table 4.20.

Table 4.19: Percentage dissolution of sulfadoxine in Fansidar® in H_2O.

Sulfadoxine (H_2O)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	28.63	25.89	24.94	29.20	27.78	27.74	27.36	5.979
20	37.00	34.49	33.64	36.83	35.40	35.43	35.47	3.676
30	40.79	38.56	37.90	40.59	39.57	39.18	39.43	2.862
45	43.35	41.73	40.73	43.08	42.36	42.08	42.22	2.252
60	44.63	43.35	43.02	44.53	43.93	43.48	43.82	1.499

Table 4.20: Percentage dissolution of pyrimethamine in Fansidar® in H_2O.

Pyrimethamine (H_2O)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	57.01	56.05	55.94	58.36	58.24	56.94	57.09	1.815
20	62.88	57.67	53.89	58.96	53.15	64.91	58.58	8.046
30	66.64	63.07	63.42	66.72	65.49	58.77	64.02	4.694
45	67.49	61.22	64.43	67.18	68.45	67.09	65.98	4.072
60	67.44	65.98	65.62	68.37	67.63	66.90	66.99	1.557

Sulfadoxine failed to comply with the requirements with 39.4% dissolved in 30 minutes and after 60 minutes still only 43.8% had dissolved. Pyrimethamine did not comply with the requirements since three of the individual samples had a dissolution percentage lower than 65% (Q+5%) in 30 minutes.

From the results obtained for the Fansidar dissolutions, it became clear that the best and most suitable dissolution medium appeared to be 0.1 N HCl as both active ingredients produced compliant but still discriminatory results. Furthermore, these results showed that maximum dissolution only occurred after 45 minutes. To compare the results obtained for each medium two graphs were created, one for sulfadoxine and the other for pyrimethamine, each illustrating the % dissolution as shown in Figure A.7 and A.8.

4.5 The dissolution testing of Falcistat®

As with Fansidar®, Falcistat® was also tested in the same three mediums, under the same conditions, using the same method. The reason for this was to compare a generic product's results to that of the innovator product in order to establish whether only the innovator (Fansidar®) produced best results with 0.1 N HCl as dissolution medium.

4.5.1 Falcistat® in pH 6.8 PBS:

The results obtained for the dissolution of sulfadoxine and pyrimethamine in PBS, are illustrated in Table 4.21 and Table 4.22.

Table 4.21: Percentage dissolution of sulfadoxine in Falcistat® in PBS (pH 6.8).

Sulfadoxine (PBS pH 6.8)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
10	65.01	67.30	65.33	71.66	64.88	65.28	66.58	3.971
20	81.41	83.14	81.74	81.64	83.16	83.81	82.48	1.221
30	85.47	88.00	88.66	83.12	87.95	89.97	87.19	2.840
45	90.22	90.55	91.35	83.55	91.78	93.25	90.12	3.759
60	90.75	90.58	92.18	83.89	92.25	94.35	90.66	3.955

Sulfadoxine complied with the requirements with 87.2% dissolved in 30 minutes and again over 60% dissolved within the first 10 minutes.

Table 4.22: Percentage dissolution of pyrimethamine in Falcistat® in PBS (pH 6.8).

Pyrimethamine (PBS pH 6.8)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	28.56	29.26	28.90	29.44	28.62	28.91	28.95	1.188
20	35.06	35.37	35.14	32.50	36.57	36.65	35.21	4.277
30	36.13	37.57	37.58	33.73	37.98	38.20	36.86	4.600
45	36.18	39.36	37.61	33.12	38.07	38.91	37.21	6.157
60	37.78	37.65	39.61	33.96	38.53	46.74	39.04	10.817

Pyrimethamine failed to comply with the requirements with 36.9% dissolved in 30 minutes and a maximum of 39% dissolved in 60 minutes.

4.5.2 Falcistat® in 0.1 N HCl:

Table 4.23: Percentage dissolution of sulfadoxine in Falcistat® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	59.81	58.12	62.61	62.93	58.87	62.53	60.81	3.503
20	77.23	78.04	80.21	81.26	77.89	79.26	78.98	1.955
30	85.83	86.72	88.57	89.30	86.80	86.52	87.29	1.534
45	92.23	92.32	93.45	94.03	93.06	91.37	92.74	1.035
60	94.89	94.49	95.17	95.80	94.79	92.45	94.60	1.210

Table 4.24: Percentage dissolution of pyrimethamine in Falcistat® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
10	82.36	85.25	87.71	89.77	83.45	86.13	85.78	3.176
20	87.77	91.37	88.57	91.09	84.76	89.00	88.76	2.729
30	90.52	92.51	92.57	92.65	90.62	88.89	91.30	1.681
45	90.77	92.74	92.63	92.94	90.95	88.96	91.50	1.707
60	91.13	92.48	89.44	92.87	91.30	89.26	91.08	1.643

Dissolution with 0.1 N HCl as medium presented remarkable results and both active ingredients complied with the requirements for dissolution testing. Sulfadoxine complied with the requirements with 87.3% dissolved in 30 minutes, 60% of which dissolved in the first 10 minutes, and pyrimethamine complied with the requirements with 91.3% dissolved in 30 minutes (note that 85.8% dissolved in the first 10 minutes).

4.5.3 Falcistat® in water:

The results obtained for this dissolution are presented in Table 4.25 and Table 4.26 below.

Table 4.25: Percentage dissolution of sulfadoxine in Falcistat® in H_2O.

Sulfadoxine (H_2O)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
10	31.38	31.12	28.81	29.35	30.16	30.17	30.16	3.280
20	37.61	36.60	35.97	36.58	36.14	37.95	36.81	2.169
30	39.76	38.83	38.94	38.90	38.16	41.10	39.28	2.608
45	41.14	40.58	40.51	40.67	39.48	42.99	40.89	2.840
60	42.01	41.46	41.23	41.61	40.28	44.07	41.78	3.022

As with Fansidar®, sulfadoxine failed to comply with the requirements with water as dissolution medium as 39.3% dissolved in 30 minutes and note that at 60 minutes still only 41.8% dissolved.

Table 4.26: Percentage dissolution of pyrimethamine in Falcistat® in H_2O.

Pyrimethamine (H_2O)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
10	61.72	53.78	48.50	51.42	50.30	0.00	44.29	50.087
20	65.30	59.76	58.36	63.82	63.44	52.64	60.55	7.731
30	67.41	59.35	62.90	70.34	55.34	60.10	62.57	8.834
45	74.12	60.66	70.43	65.09	55.17	62.94	64.74	10.523
60	71.98	67.69	62.89	66.08	66.37	73.98	68.17	6.012

Pyrimethamine too failed to comply with the requirements as four of the individual samples had a dissolution percentage lower than 65% (Q+5%) in 30 minutes.

The results obtained for Falcistat® presented an identical scenario to that of Fansidar®. The percentage dissolution for each of the mediums is illustrated with graphs for both sulfadoxine and pyrimethamine in Figure A.9 and A.10.

4.6 Stability test dissolutions

From the results obtained with the dissolution tests of Fansidar® and Falcistat® in the three different media, it was clear that 0.1 N HCl would be the medium of choice for the remainder of this study as this was the only medium in which both active ingredients passed the dissolution requirements.

Table 4.27: Stability test dissolution results for sulfadoxine and pyrimethamine.

	Sulfadoxine (µg/ml)				*Pyrimethamine (µg/ml)*			
	D 1	**D 2**	**D 3**	**D 4**	**D 1**	**D 2**	**D 3**	**D 4**
Run 1	211.19	439.06	210.43	460.87	25.82	26.04	23.78	25.09
Run 2	210.28	435.85	209.34	462.54	25.41	25.80	23.55	24.97
Run 3	208.54	437.13	209.88	461.96	24.93	25.55	23.53	24.97
Run 4	209.64	435.61	209.60	461.81	24.91	25.60	23.26	24.92
Run 5	209.52	434.32	208.97	463.07	24.89	25.46	23.23	24.89
Run 6	209.31	434.09	209.34	462.77	24.98	25.45	23.06	24.70
Run 7	210.06	433.90	209.12	461.96	24.96	25.47	23.18	24.52
Run 8	210.85	436.18	210.34	463.01	25.04	25.51	23.27	24.78
Run 9	210.60	434.51	209.98	462.39	25.04	25.61	23.52	24.77
Run 10	210.20	433.23	210.26	463.27	24.92	25.56	23.08	24.75
Run 11	209.68	433.73	210.07	462.85	25.07	25.64	23.40	24.79
Run 12	209.98	433.51	209.27	460.43	25.25	25.31	23.80	24.77
Av.	**210.00**	**435.09**	**209.72**	**462.24**	**25.10**	**25.58**	**23.39**	**24.83**

Since the stability of both sulfadoxine and pyrimethamine in 0.1 N HCl were questioned, a stability test dissolution was performed. D 1 and D 2 in the table above represent one Fansidar® tablet in 0.01 N HCl and one in 0.1 N HCl, while D 3 and D 4 represent one Falcistat® tablet in 0.01 N HCl and one in 0.1 N HCl, respectively. These samples were injected twelve times (Run 1 – 12) on an HPLC system and the % RSDs for the standards of both sulfadoxine and pyrimethamine were 0.43 and 2.23, respectively.

Note that, even though there wasn't a remarkable difference between the concentrations obtained for pyrimethamine, sulfadoxine produced another scenario. Not even 50% (250 µg/ml) sulfadoxine dissolved in 0.01 N HCl for either Fansidar® or Falcistat®, whilst 0.1 N HCl remains the medium of choice since it, once again, produced best results. Furthermore, neither one of

the two active ingredients' stability deteriorated significantly during this test as shown in Figures A.11 to A.14.

4.7 The dissolution testing of generic products

4.7.1 Laridox®

Table 4.28: Percentage dissolution of sulfadoxine in Laridox® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	86.79	80.92	85.14	86.10	84.04	85.90	84.82	2.508
45	91.82	90.06	92.37	93.00	87.71	90.07	90.84	2.144

Table 4.29 Percentage dissolution of sulfadoxine in Laridox® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	101.00	100.14	100.26	98.80	94.24	97.15	98.60	2.568
45	99.88	98.96	100.06	100.00	86.00	87.31	95.37	7.105

Sulfadoxine and pyrimethamine in Laridox® complied with the requirements with 84.8% and 98.6% dissolved in 30 minutes, respectively as illustrated in Table 4.28 and Table 4.29.

4.7.2 Orodar®

Table 4.30: Percentage dissolution of sulfadoxine in Orodar® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	79.51	80.89	81.14	82.22	84.43	82.08	81.71	2.021
45	88.77	89.11	88.17	90.44	92.86	89.07	89.73	1.895

Table 4.31: Percentage dissolution of pyrimethamine in Orodar® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
30	93.23	89.22	92.73	94.30	99.54	95.91	94.15	3.659
45	94.70	94.02	94.01	96.93	99.03	97.83	96.09	2.227

Orodar® complied with the requirements with 81.7% dissolved in 30 minutes and 94.2% dissolved in 30 minutes for sulfadoxine and pyrimethamine, respectively as shown in Table 4.30 and Table 4.31.

4.7.3 Tansidar®

Table 4.32: Percentage dissolution of sulfadoxine in Tansidar® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
30	26.58	14.33	16.12	20.59	20.40	16.99	19.17	22.861
45	41.83	34.35	33.83	39.88	33.12	28.46	35.24	13.793

Table 4.33: Percentage dissolution of pyrimethamine in Tansidar® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
30	41.49	31.06	32.34	34.83	30.13	30.95	33.47	12.725
45	58.91	53.36	53.16	57.41	47.23	47.41	52.92	9.219

Tansidar® on the other hand failed to comply with the requirements as only 19.2% dissolved in 30 minutes for sulfadoxine and 33.5% dissolved in 30 minutes for pyrimethamine. Also note that the % RSDs are much higher than with Laridox® and Orodar®.

4.7.4 Sulfadoxine & Pyrimethamine Tablets USP

Table 4.34: Percentage dissolution of sulfadoxine in Sulfadoxine & Pyrimethamine Tablets USP in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	79.83	81.00	65.87	82.24	81.74	78.16	78.14	7.918
45	87.87	83.15	84.80	90.55	85.16	83.33	85.81	3.354

Table 4.35: Percentage dissolution of pyrimethamine in Sulfadoxine & Pyrimethamine Tablets USP in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	95.30	95.91	76.78	98.73	94.04	91.48	92.04	8.519
45	95.56	91.06	89.99	99.43	92.56	92.04	93.44	3.730

The Sulfadoxine & Pyrimethamine Tablets USP complied with the requirements as 78.1% sulfadoxine dissolved in 30 minutes and 92.0% pyrimethamine dissolved in 30 minutes.

4.7.5 Sulphadar®

Table 4.36: Percentage dissolution of sulfadoxine in Sulphadar® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	93.51	102.13	101.97	69.72	71.48	85.97	87.46	16.447
45	98.52	88.79	99.56	76.32	76.44	103.04	90.45	13.133

Table 4.37: Percentage dissolution of pyrimethamine in Sulphadar® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	78.08	87.43	86.10	82.20	83.37	68.08	80.88	8.738
45	79.91	68.30	79.87	87.23	88.68	82.01	81.00	8.947

Sulphadar® also complied with the requirements with 87.5% dissolved in 30 minutes and 80.9% dissolved in 30 minutes for sulfadoxine and pyrimethamine, respectively.

4.7.6 Malostat®

Table 4.38: Percentage dissolution of sulfadoxine in Malostat® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	98.57	101.65	91.82	97.91	82.44	100.29	95.45	7.554
45	103.79	102.14	81.34	96.29	99.08	100.42	97.18	8.411

Table 4.39: Percentage dissolution of pyrimethamine in Malostat® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	96.81	99.16	91.98	95.31	80.46	100.88	94.10	7.825
45	100.92	98.14	78.60	93.62	95.55	96.21	93.84	8.383

Both sulfadoxine and pyrimethamine in Malostat® complied with the requirements with 95.4% and 94.1% dissolved in 30 minutes, respectively.

4.7.7 Dionsdar®

Table 4.40: Percentage dissolution of sulfadoxine in Dionsdar® in 0.1 N HCl.

Sulfadoxine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
30	65.41	57.88	66.07	63.50	60.04	60.33	62.20	5.276
45	74.41	74.29	74.77	69.66	69.02	50.50	68.77	13.526

Table 4.41: Percentage dissolution of pyrimethamine in Dionsdar® in 0.1 N HCl.

Pyrimethamine (0.1 N HCl)								
Time (min)	% Dissolution						Mean Diss.	% R.S.D.
	(1)	(2)	(3)	(4)	(5)	(6)		
0	0	0	0	0	0	0	0	0
30	98.61	83.71	96.43	100.04	94.47	96.15	94.90	6.135
45	100.06	99.77	102.71	97.15	95.33	70.13	94.19	12.803

Sulfadoxine in Dionsdar® failed to comply with the requirements with 62.2% dissolved in 30 minutes. At 45 minutes however, 68.8% sulfadoxine had dissolved which stresses the fact that maximum dissolution only occurs after 45 minutes. Pyrimethamine however complied with the requirements with 94.9% dissolved in 30 minutes. Had Dionsdar® been inadequately formulated, both active ingredients would have failed to comply with the requirements for dissolution testing and since the active ingredient that was investigated during this study was pyrimethamine, it was decided that the dissolution test for Dionsdar® would not be repeated as pyrimethamine complied with the dissolution requirements.

4.8 The dissolution testing of Tansidar® in PBS

Since both sulfadoxine and pyrimethamine in Tansidar® failed to meet the USP requirements for dissolution with 0.1 N HCl as medium, it was decided that a dissolution test should be performed in PBS (pH 6.8) in order to compare results.

Table 4.42: Percentage dissolution of sulfadoxine in Tansidar® in PBS (pH 6.8).

Sulfadoxine (PBS pH 6.8)								
Time (min)	**% Dissolution**						**Mean Diss.**	**% R.S.D.**
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**		
0	0	0	0	0	0	0	0	0
30	31.41	31.47	27.54	32.25	26.69	29.65	29.83	7.662
45	40.55	39.99	36.00	41.06	34.23	40.47	38.72	7.400

Sulfadoxine in Tansidar® once more failed to comply with the dissolution requirements with 29.8% dissolved in 30 minutes while pyrimethamine didn't undergo dissolution as no significant concentrations were produced. The comparison between the two dissolution tests performed for Tansidar® is illustrated in Figure A.15.

CHAPTER 5

SUMMARY AND CONCLUSION

In any industry today, quality is of great importance, and even more so in the case of the pharmaceutical industry. It is vital that pharmaceutical products meet the criteria of international pharmacopoeias such as the USP prior to the introduction of these products to the public. Therefore every product that claims pharmacopoeial quality needs to comply with the acceptance criteria specified in the product monographs of these pharmacopoeias.

Two other aspects that share the importance of quality are safety and efficacy. A pharmaceutical product should efficiently aid in the treatment of disease and not pose a threat to human life, nor should it cause major adverse effects that might compromise the quality of life. In actual fact, these products should improve life quality.

Disease and its treatment are as ancient as life itself and still certain illnesses cause major problems globally. One such a disease is malaria caused by *P. falciparum*, which is responsible for the majority of fatalities in Africa. Fansidar® is one of the many antimalarial drugs available to combat this disease and is used as second-line treatment of malaria.

Fansidar® contains two active ingredients; sulfadoxine (500 mg) and pyrimethamine (25 mg). There seems to be a problem when performing a dissolution test of this product, as neither the innovator product nor 90% of its generics comply with the USP requirements for dissolution with regard to the pyrimethamine. The USP prescribed dissolution medium is in question and believed not to be the ideal medium for the combination drug. This sets the aim and the focus for this study.

The following objectives were set:

- To investigate and recommend a dissolution medium that is more suitable in terms of discriminating power than the dissolution medium of the USP prescribed for the dissolution testing of sulfadoxine and pyrimethamine combination products.
- To amend the HPLC method utilized by the USP for the analysis of dissolution samples of sulfadoxine and pyrimethamine combination products, in order to enhance the robustness and selectivity of the HPLC method.

- To investigate the chemical stability of the dissolution samples of sulfadoxine and pyrimethamine combination products, as a function of dissolution medium and analytical run time.

The dissolution tests of Fansidar® were performed successfully in three different media (PBS pH 6.8, 0.1 N HCl and water) with Method B as analytical method of choice. With PBS as dissolution medium, sulfadoxine complied with the requirements with 89.7% dissolved in 30 minutes while pyrimethamine failed the requirements with 35.7% dissolved in 30 minutes. However, using 0.1 N HCl as dissolution medium, both sulfadoxine and pyrimethamine complied with the requirements with 82.3% and 99.6% dissolved in 30 minutes, respectively. This emphasizes the statement that PBS is not a suitable dissolution medium for the combination tablet. The last dissolution test was performed with water as dissolution medium and pyrimethamine failed to comply with the requirements with 64% dissolved in 30 minutes and sulfadoxine too failed to comply with the requirements with 39.4% dissolved in 30 minutes. The dissolution medium of choice was 0.1 N HCl as both active ingredients complied with the requirements for dissolution testing with this medium.

A generic product, Falcistat® was successfully tested in the same three media as with the innovator product Fansidar®. Using PBS as dissolution medium, sulfadoxine complied with the requirements with 87.2% dissolved in 30 minutes while pyrimethamine failed to comply with requirements with 36.9% dissolved in 30 minutes. Dissolution testing with 0.1 N HCl produced the best results as both sulfadoxine and pyrimethamine complied with the requirements with 87.3% and 91.3% dissolved in 30 minutes, respectively. As previously, sulfadoxine failed to comply with the requirements with 39.3% dissolved in 30 minutes using water as medium, as did pyrimethamine with 62.6% dissolved in 30 minutes. Both sulfadoxine and pyrimethamine complied with the requirements for dissolution testing with 0.1 N HCl as dissolution medium, making it the medium of choice as it was the only medium where both actives complied with the criteria.

Seven other generic products were successfully tested using Method B and 0.1 N HCl as dissolution medium. Laridox®, Orodar®, Sulphadar®, Malostat® and Sulfadoxine & Pyrimethamine Tablets USP all complied with the requirements for dissolution testing with 0.1 N HCl for both sulfadoxine and pyrimethamine whilst with Dionsdar® pyrimethamine complied and sulfadoxine didn't comply with requirements as 62.2% dissolved in 30 minutes. However it was noted that at 45 minutes 68.8% sulfadoxine dissolved. Tansidar® failed to comply with the requirements on both accounts. Hence, another dissolution test of Tansidar® was performed, this time using PBS as dissolution medium (the USP dissolution medium) and sulfadoxine failed to comply with the dissolution requirements whilst pyrimethamine didn't undergo dissolution. The reason for this might be that the composition of the Tansidar® tablets is incorrect or faulty.

A new HPLC method was successfully developed, using the method stated in the USP as foundation with an adjusted pH of the mobile phase. The mobile phase of this newly developed method was now more alkaline, forcing the pyrimethamine peak to appear after the sulfadoxine peak. This prevents impurities of the larger sulfadoxine peak to interfere with that of the pyrimethamine peak. Therefore the results obtained using the altered mobile phase was more accurate and a more robust and selective HPLC method was developed.

At this stage three media (0.1 N HCl, PBS pH 6.8 and the original USP mobile phase) were tested with the original USP method (Method A) and the new method of which the mobile phase was altered (Method B). With Method A both sulfadoxine and pyrimethamine's single components obtained the lowest % RSD with PBS whilst for the combination, both obtained the lowest % RSD with 0.1 N HCl. The overall % RSD for sulfadoxine and pyrimethamine with Method A was 0.47 and 22.23, respectively. Testing the three media with Method B produced a different scenario; sulfadoxine's single component produced the lowest % RSD with 0.1 N HCl and in combination obtained the lowest % RSD with PBS. Pyrimethamine on the other hand obtained the lowest % RSD with mobile phase in combination and as single component. The overall % RSD for sulfadoxine and pyrimethamine with Method B was 0.40 and 15.78 respectively. Hence, pyrimethamine produced a remarkably smaller %RSD with Method B and validation proceeded for this method.

Stability test dissolutions were performed successfully on both Fansidar® and Falcistat®, using two different concentrations of HCl (0.01 N and 0.1 N). There wasn't a remarkable difference between the concentrations obtained for pyrimethamine of the two products in both 0.01 N and 0.1 N HCl. However, this wasn't the case for sulfadoxine as not even 50% (250 µg/ml) dissolved in the 0.01 N HCl for either Fansidar® or Falcistat®. Once more 0.1 N HCl proved to be the medium of choice as it produced best results with the stability test dissolutions and neither sulfadoxine nor pyrimethamine's stability deteriorated significantly.

During this study it became evident that the USP dissolution method for sulfadoxine-pyrimethamine combination tablets, in specific the dissolution medium and the mobile phase used in this method should be revised. Evidence proved that a more alkaline mobile phase (pH 4) as well as a different dissolution medium (0.1 N HCl) accounted for better results as the majority of the products including the innovator product, complied with the dissolution requirements specified by the USP. Furthermore, it is suggested that the dissolution time of this specific dissolution test should be prolonged as it was proved that maximum dissolution occurred after 45 minutes.

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APPENDIX 1

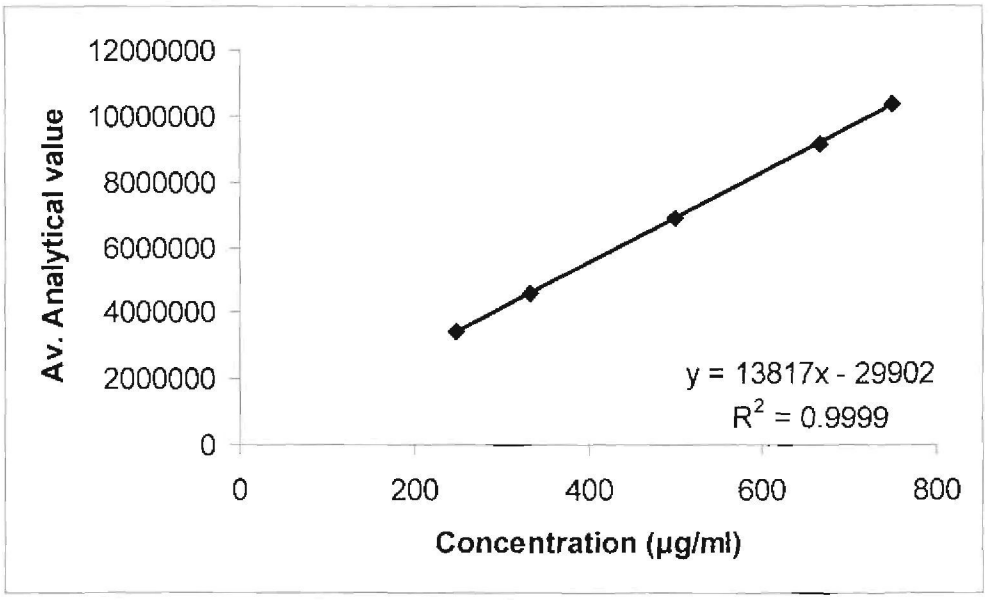

Figure A.1: Regression for the validation of sulfadoxine in PBS (pH 6.8).

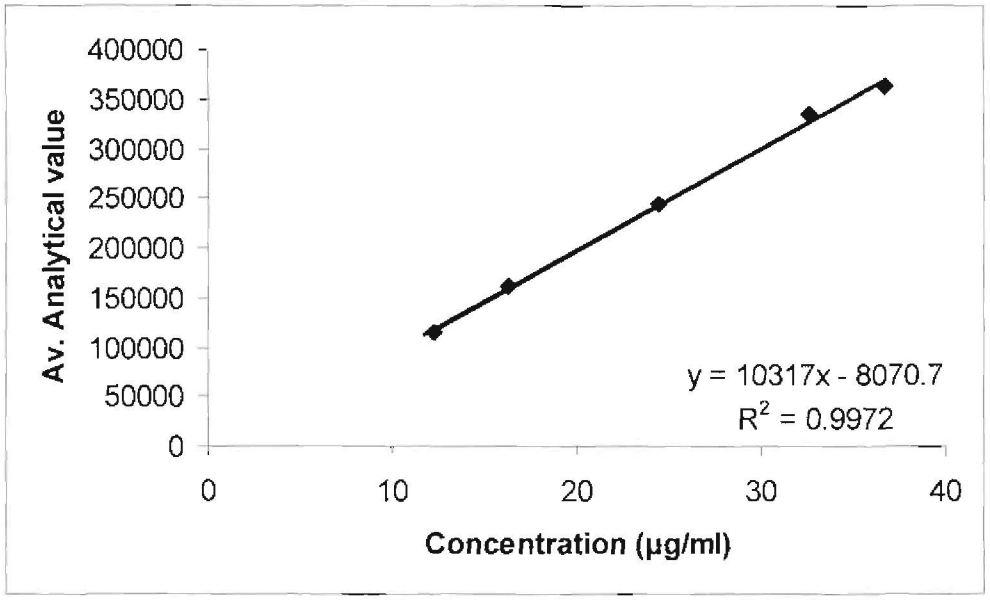

Figure A.2: Regression for the validation of pyrimethamine in PBS (pH 6.8).

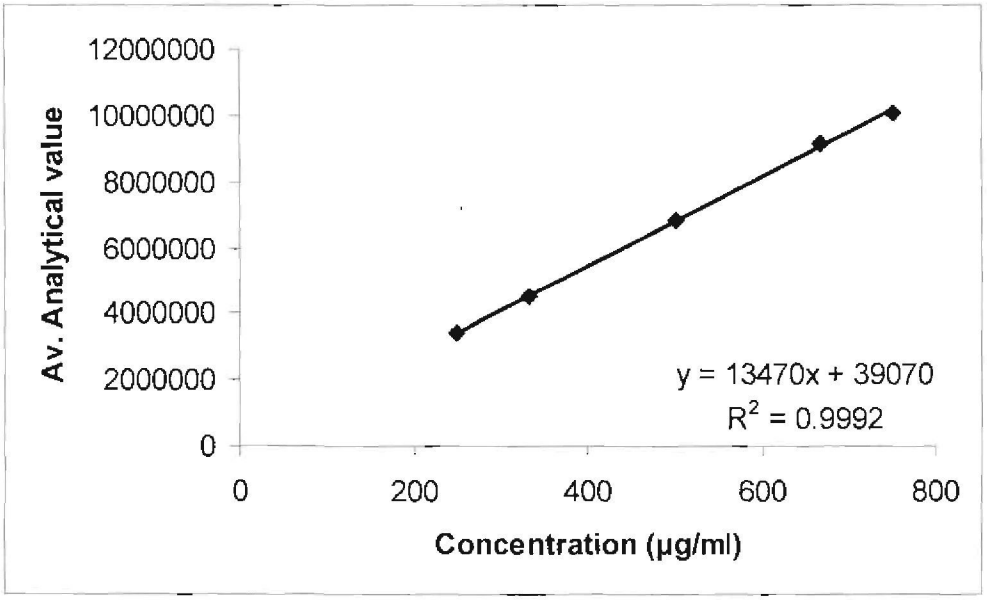

Figure A.3: Regression for the validation of sulfadoxine in 0.1 N HCl solution.

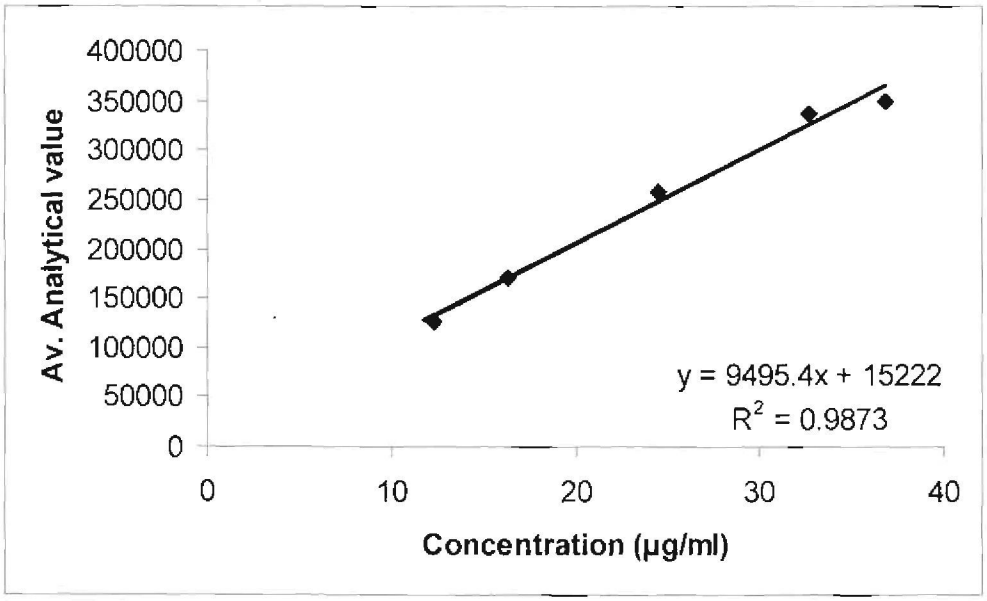

Figure A.4: Regression for the validation of pyrimethamine in 0.1 N HCl solution.

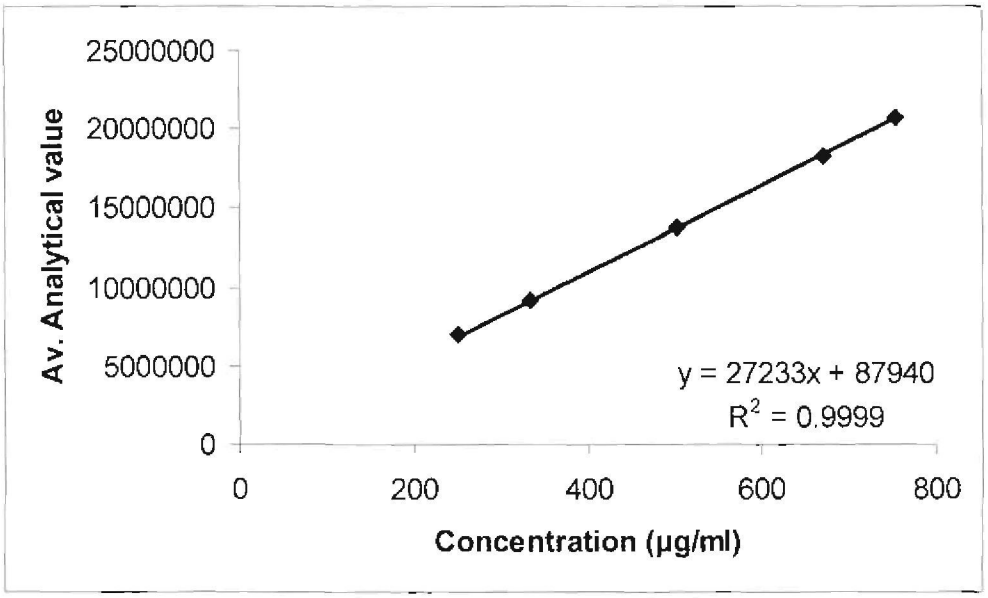

Figure A.5: Regression for the validation of sulfadoxine in distilled water.

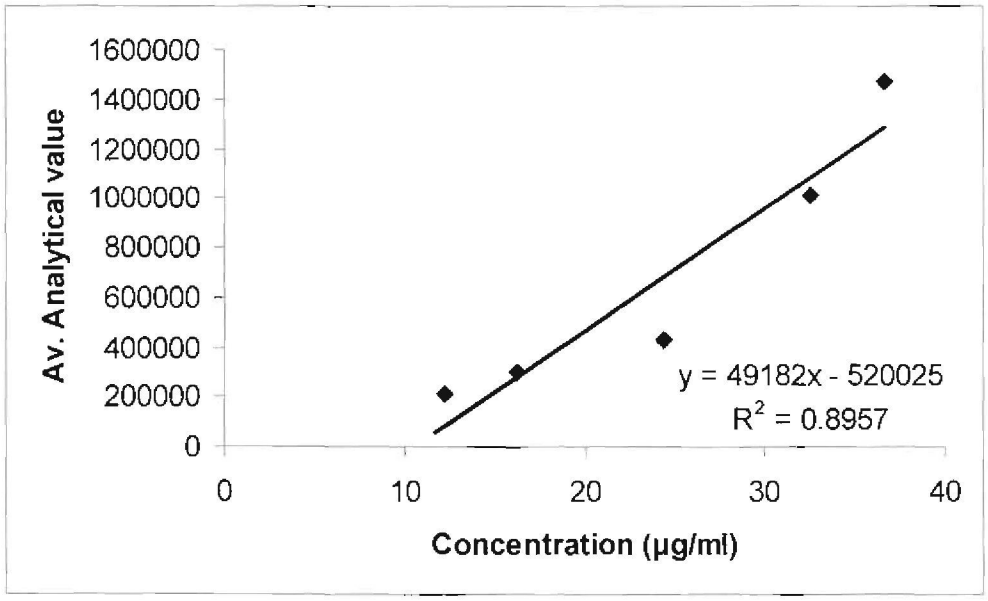

Figure A.6: Regression for the validation of pyrimethamine in distilled water.

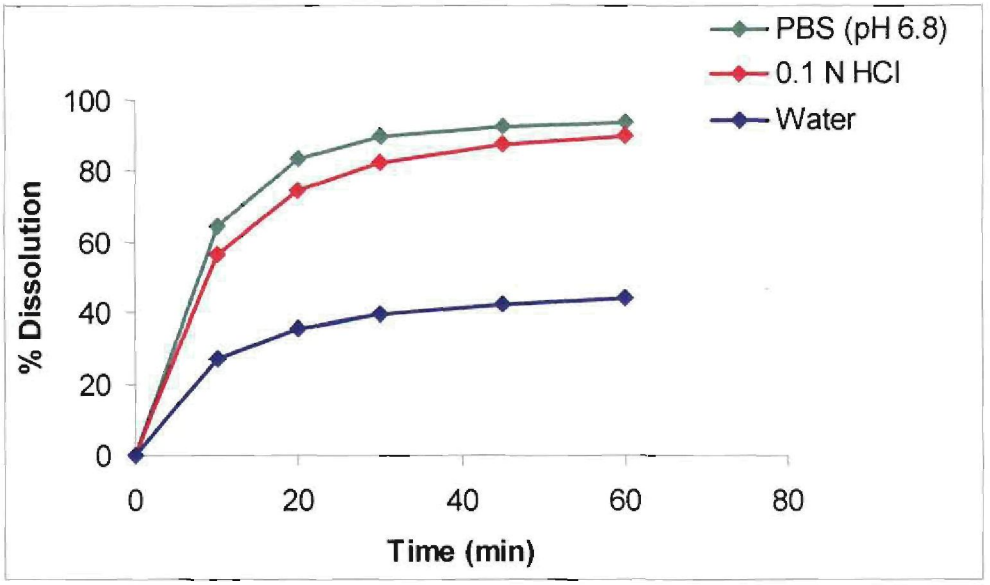

Figure A.7: The dissolution profile of sulfadoxine in Fansidar® with three different media.

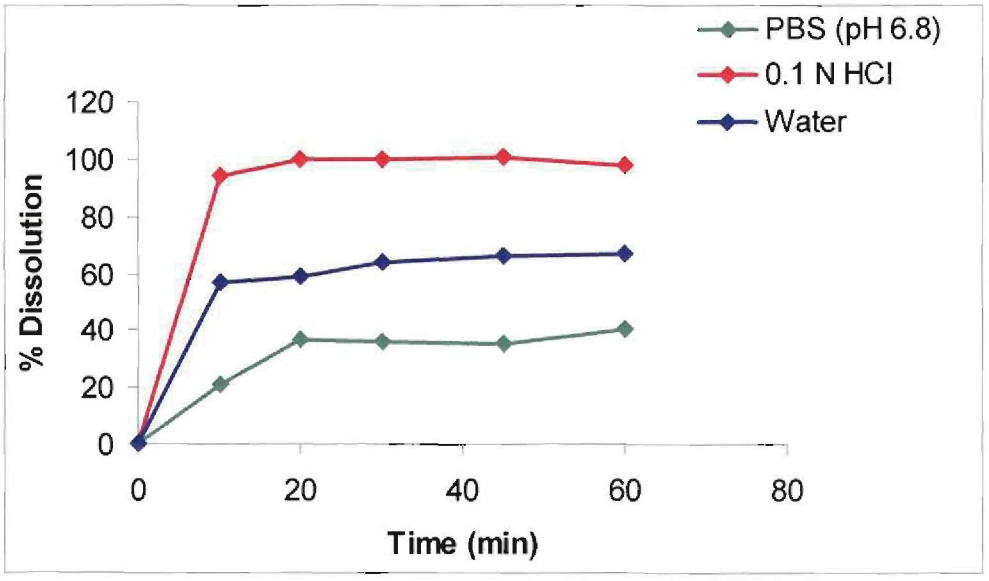

Figure A.8: The dissolution profile of pyrimethamine in Fansidar® with three different media.

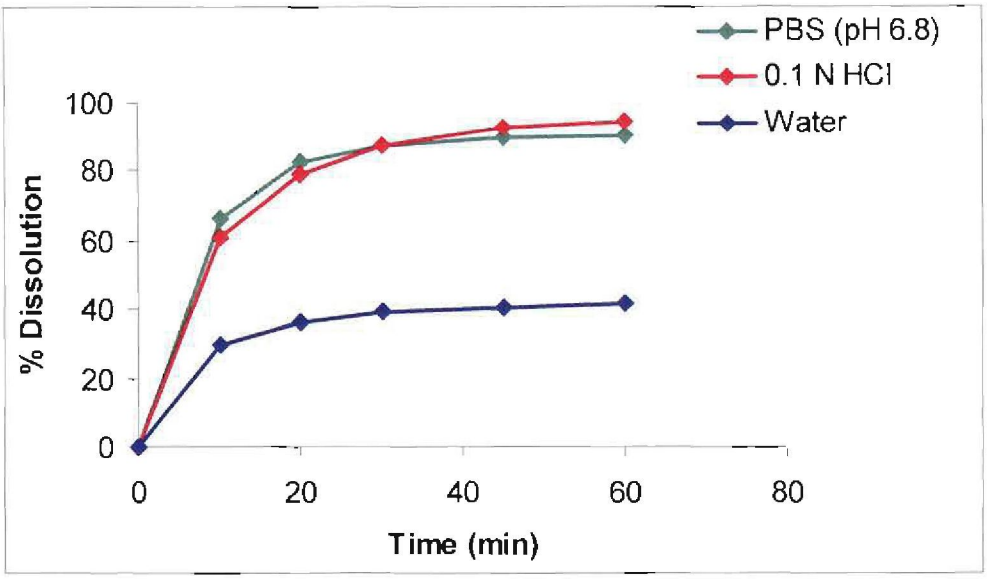

Figure A.9: The dissolution profile of sulfadoxine in Falcistat® with three different media.

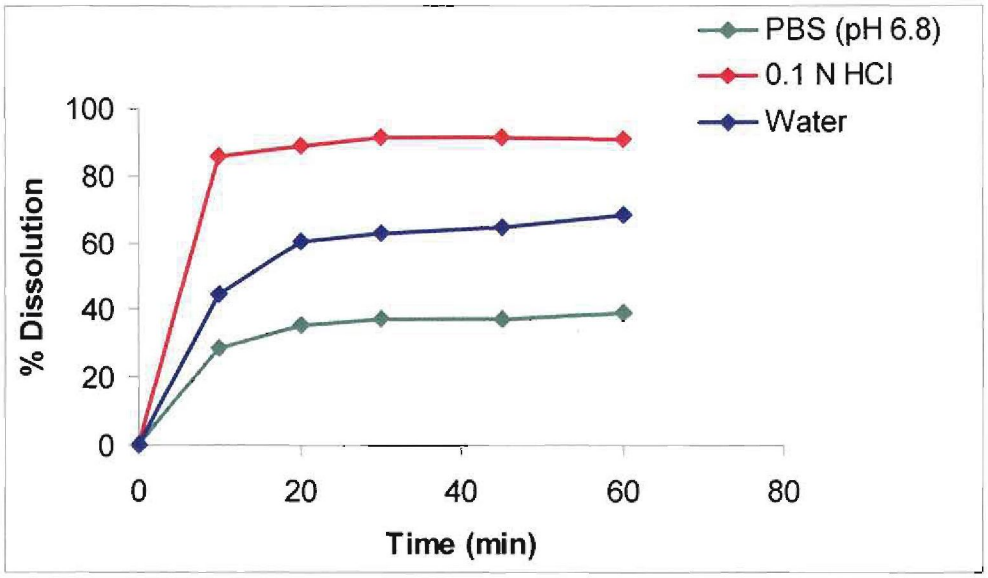

Figure A.10: The dissolution profile of pyrimethamine in Falcistat® with three different media.

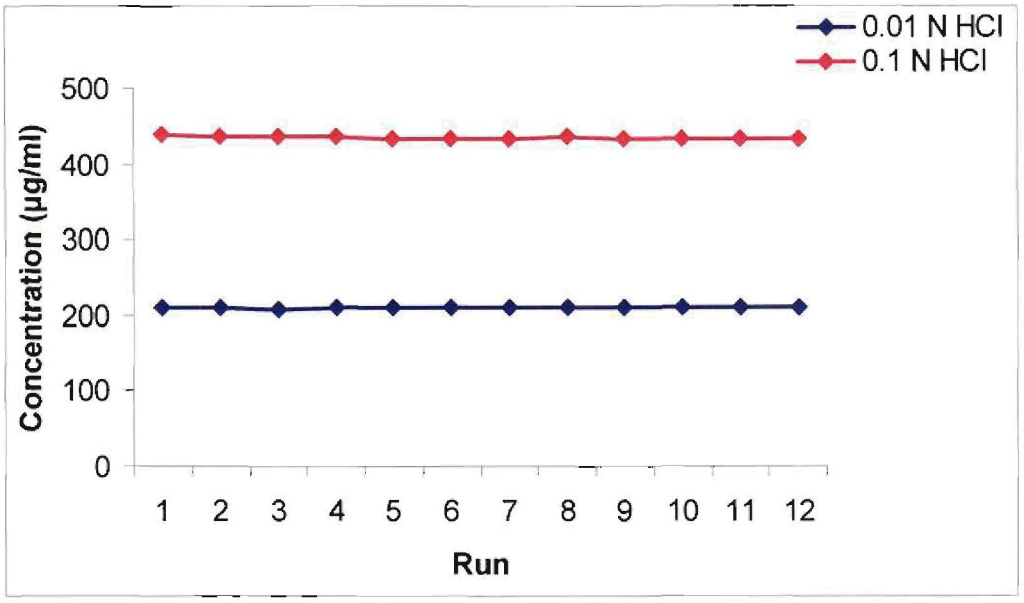

Figure A.11: Stability test dissolution of sulfadoxine in Fansidar® with HCl.

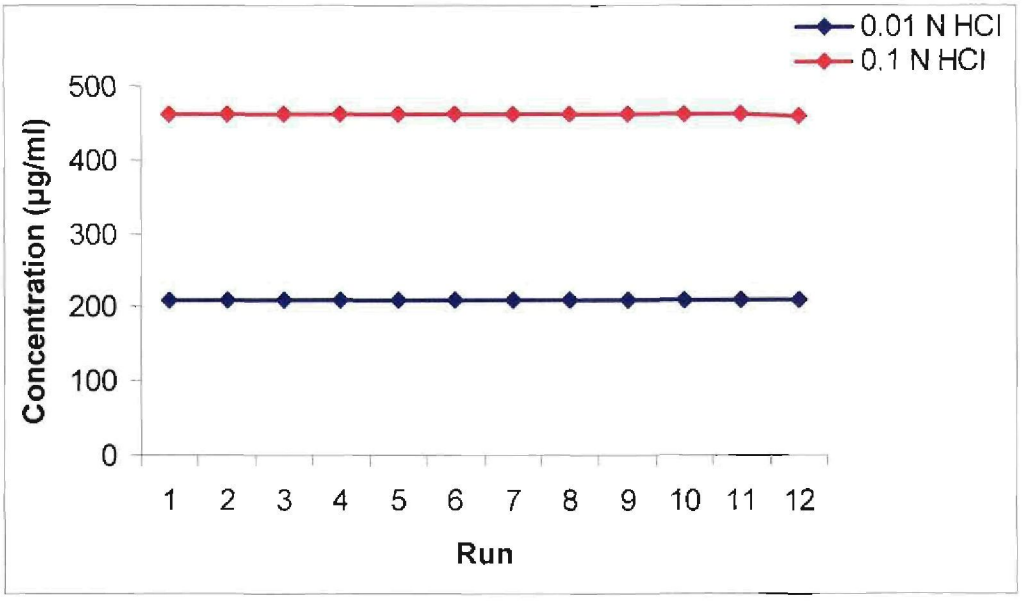

Figure A.12: Stability test dissolution of sulfadoxine in Falcistat® with HCl.

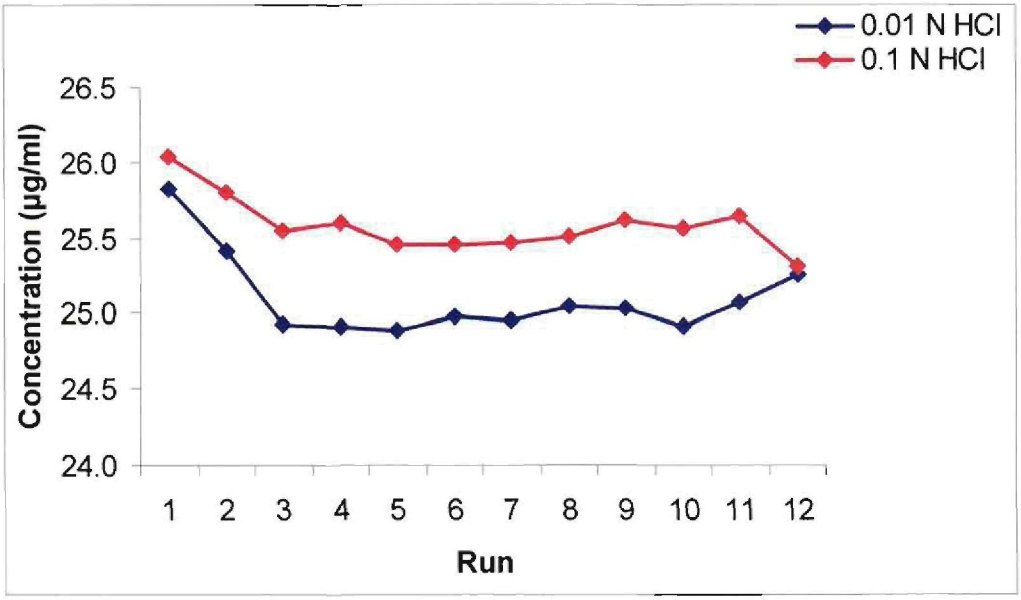

Figure A.13: Stability test dissolution of pyrimethamine in Fansidar® with HCl

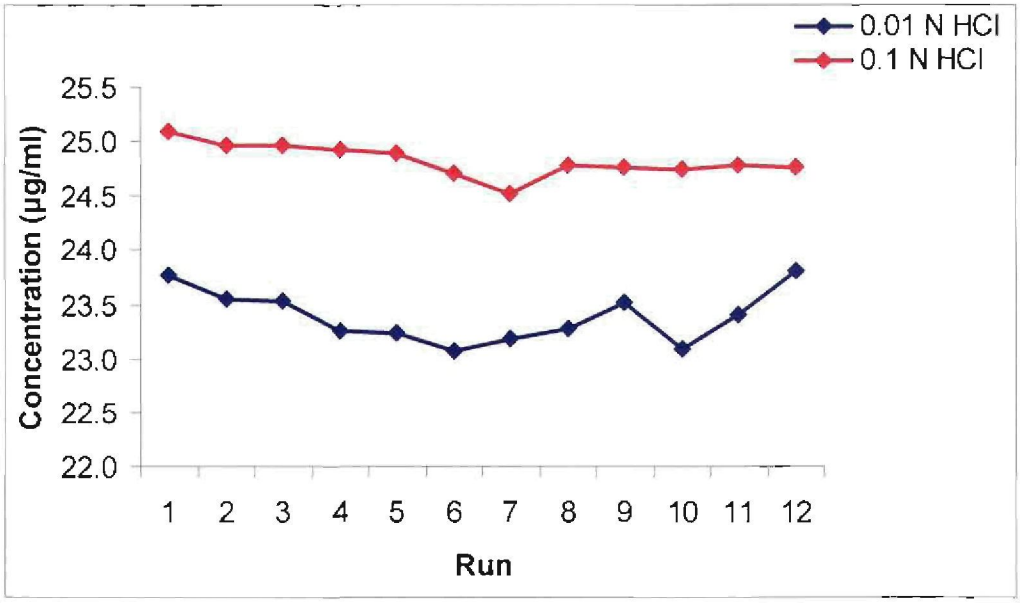

Figure A.14: Stability test dissolution of pyrimethamine in Falcistat® with HCl

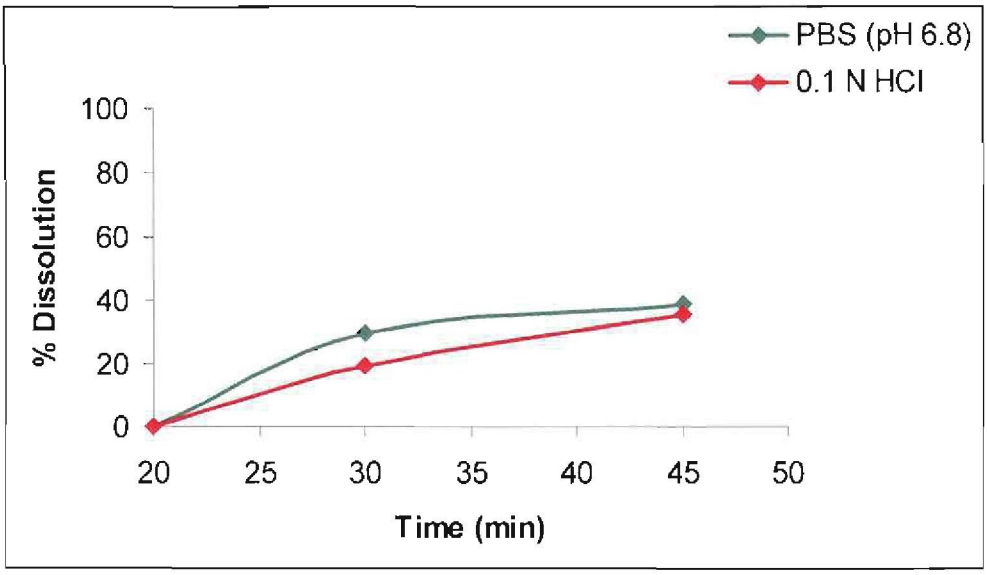

Figure A.15: The dissolution profile of sulfadoxine in Tansidar® as tested in PBS (pH 6.8) and 0.1 N HCl.