# Solubility and dissolution testing of selected sulfadoxine/pyrimethamine mixtures

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*This dissertation is dedicated to my parents Nico and Karin Holtzkamp*

**“SOMETIMES THE BRAVEST THING YOU CAN DO IS TO KEEP GOING WHEN YOU REALLY FEEL LIKE GIVING UP.”**

UNKNOWN

## ABSTRACT

Although malaria is an age old disease it continues to plague mankind especially in the African regions. Pregnant women are more likely to be infected with malaria due to the hormonal changes with more severe symptoms and outcomes.

Intermittent preventive therapy with sulfadoxine (S) and pyrimethamine (P) are considered to be an effective way of preventing malaria in pregnant women; but the increase of the resistance of the malaria parasite to sulfadoxine and pyrimethamine is still a major concern. Some of the possible causes of resistance include the poor solubility and dissolution rate of both drugs. Addressing these problems might be a positive stepping stone towards combating malaria resistance in the future.

The focus of this study was to determine the solubility and dissolution properties and possible chemical interactions in the powder mixtures compared to the single components. Distilled water, phosphate buffer (pH 6.8) and 0.1 N HCl were used as media for solubility and dissolution testing.

The results of the SP combinations emphasised that sulfadoxine and pyrimethamine is more soluble in distilled water and PBS; but when in combination both of these actives' solubility decreases in 0.1 N HCl. In contrast with the solubility results, the best results obtained during dissolution testing were in 0.1 N HCl. For each dissolution medium, only some of the SP combinations correspond with the USP requirements (60% or higher dissolution in 30 minutes) for each tablet.

Differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD) were used to establish if interactions occur in the powder mixtures. The DSC results showed that during heating of certain SP combination ratios, shifting of melting point and even melting point depression occurs. This may indicate the possibility of a eutectic mixture being formed. With a percentage pyrimethamine of 55% (w/w) or higher in the mixture, two distinguishable melting endotherms were visible. XRPD results indicated that during exposure of SP combinations to distilled water, no other solid-state forms such as co-crystals of sulfadoxine and pyrimethamine formed.

To conclude, there is definitely an increase in the solubility and dissolution rate of sulfadoxine and pyrimethamine when in combination. The significance and origin of the increased solubility requires further investigation. The possibility of a eutectic mixture being formed also warrants further investigation.



Keywords: sulfadoxine, pyrimethamine, solubility, physico-chemical properties

## OPSOMMING

Alhoewel malaria ‘n eeue-oue siekte is, word dit steeds as ‘n gesondheidsprobleem gesien, veral in die Afrika-streke. Die risiko vir malaria in swanger vrouens is baie hoër as gevolg van die hormonale veranderinge en dus veroorsaak dit dat erger simptome ervaar word.

Sulfadoksien (S) en pirimetamien (P) word gebruik as profilakse teen malaria in swanger vrouens en is steeds ‘n effektiewe kombinasie om malaria te voorkom, maar die toename in weerstand van die malariaparasiet teen sulfadoksien en pirimetamien bly ‘n groot bekommernis. Van die moontlike oorsake van die toename in weerstand kan as gevolg van die swak oplosbaarheid en dissolusietempo van beide sulfadoksien en pirimetamien wees. Deur hierdie probleme aan te spreek sal dit moontlik ‘n positiewe uitweg wees om malaria in die toekoms te bestry.

Die fokus van die studie was om die oplosbaarheid- en dissolusie-eienskappe asook die moontlike chemise interaksies van die mengsels met die enkel komponente te vergelyk. Gedistilleerde water, fosfaatbuffer (pH 6.8) en 0.1 N soutsuur was as mediums vir beide die oplosbaarheids- en dissolusiestudies gebruik.

Die resultate van die SP kombinasies beklemtoon die feit dat sulfadoksien en pirimetamien albei goed in PBS en water oplosbaar is, maar swak in 0.1 N HCl. Die oplosbaarheids- en dissolusie resultate kontrasteer wel mekaar. Sulfadoksien en pirimetamien het ‘n hoër dissolusie persentasie in 0.1 N HCl bereik, maar swakker persentasies in PBS en water. In elke dissolusiemedium het slegs ‘n paar van die SP kombinasies aan die USP vereistes (60% binne 30 minute) vir die SP tablet voldoen.

Differensiëleskanderingskalorimetrie (DSC) en x-straaldiffraksie (XRPD) is gebruik om enige interaksies tussen die mengsels te bepaal.

Die DSC resultate dui daarop dat by sekere verhoudings van sulfadoksien en pirimetamien slegs een endoterm vorm. Indien pirimetamien ‘n persentasie van 55% (m/m) of hoër bereik, vorm daar twee endoterms. Die XRPD resultate dui daarop dat daar geen verskuiwing of vorming van nuwe diffraksiepieke is nie en dat daar geen rekristallisasie vir beide van die geneesmiddels plaasgevind het nie.

‘n Definitiewe toename in die oplosbaarheid en dissolusietempo van sulfadoksien en pirimetamien in kombinasie is gesien. Verdere ondersoek moet ingestel word om die oorsprong van die verhoogde oplosbaarheid en dissolusietempo te bepaal. Die moontlike vorming van ‘n eutektiese mengsel regverdig verdere ondersoek.



Sleutelwoorde: sulfadoksien, pirimetamien, fisiese-chemiese eienskappe

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## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## ABBREVIATIONS

| | |
|---|---|
| **BC** | before Christ |
| **BP** | Brithish Pharmacopoeia |
| **CDC** | Centers for Disease Control |
| **PABA** | para-amino benzoicacid |
| **DHFR** | dihydrofolatereductase inhibitor |
| **DEET** | N,N-diethyl-m-toluamide |
| **DHPS** | dihydropteroate synthase |
| **DNA** | deoxyribonucleic acid |
| **DSC** | Differential Scanning Calorimetry |
| **FDC** | fixed-dose combination |
| **HIV** | human immunodeficiency virus |
| **HPLC** | high performance liquid chromatograph |
| **INT's** | insecticide-treated nets |
| **IPTp** | intermitted preventive therapy |
| **IPTp-SP** | intermitted preventive therapy - sulfadoxine/pyrimethamine |
| **P** | pyrimethamine |
| **PBS** | phosphate buffer |
| **P.*falciparum*** | Plasmoduim falciparum |
| **Ph.Int** | International Phamacopoeia |

| | |
|---|---|
| **RBS** | red blood cells |
| **% RSA** | percentage relative standard deviation |
| **S** | sulfadoxine |
| **SAMF** | South African Medicine Formulary |
| **SP** | sulfadoxine/pyrimethamine |
| **STD** | standard solution |
| **TNF α** | tumour necrosis factor |
| **UNICEF** | Children's Rights & Emergency Relief Organization |
| **USP** | Unites States Pharmacopoeia |
| **XRPD** | x-ray powder diffraction |
| **WHO** | World Health Organization |

# CHAPTER 1

# INTRODUCTION AND OBJECTIVES

## 1.1 Introduction

Malaria, an infectious disease, is caused by a single-cell parasite belonging to the Plasmodium genus. Only four species (*falciparum, vivax, ovale and malariae*) are responsible for malaria in humans and although it is an age old disease it continues to plague mankind especially in the African region (Saifi *et al.*, 2013:148).

In pregnant women, malaria is a major concern as the symptoms are more severe resulting in elevated rates of premature delivery, miscarriages, severe anaemia and low-birth-weight in neonates. By employing preventive measures such as treated bed nets, educational outreach programs and appropriate intermittent preventive treatment therapy, these side-effects can be minimised (Schantz-Dunn *et al*., 2009:190).

The WHO recommends that pregnant women must be protected against malaria infection at all times. Chloroquine has been the most effective drug in preventing infection; however over the years *Plasmodium falciparum* became more resistant to chloroquine worldwide. Sulfadoxine (S) and pyrimethamine (P), a fixed dose combination (FDC), are used as an antimalarial prophylactic drug in pregnant women during the second and third trimester. It not only protects the mother and infant from malaria infections, but also decreases the potential of foetal anaemia and low birth weight. Although the resistance of the parasite to SP is rising, the WHO still recommends that all pregnant women receive three or more doses of SP as intermittent preventive therapy (IPTp-SP) (Adam *et al.,* 2006:7; Rogawski *et al*., 2012:1096; WHO, 2015:102).

Thus, a major concern regarding that these two drugs as IPTp-SP is the constant increase of resistance of the malaria parasite to SP and the little information regarding the physico-chemical properties, solubility and permeability. Modification of these properties are possible if more information regarding these properties of SP are obtained and thus the possibility of higher therapeutic effectiveness and lower resistance to IPTp-SP. For this study, the aim and objectives were set out to obtain more information regarding the physico-chemical properties of SP and the possible increase of the solubility and dissolution of SP.

## 1.2 Aim and Objectives

The purpose of this study was to investigate the solubility and dissolution properties of various selected sulfadoxine and pyrimethamine combinations since previous unpublished work did in our laboratories indicate the enhancement of the solubility properties of both drugs when mixed together.

The following objectives were set:

- Prepare the different sulfadoxine and pyrimethamine combinations;
- Capsuling the mixtures in hard gelatine shells;
- Investigate the solubility of the mixtures using distilled water, phosphate buffer (PBS, pH 6.8) and 0.1 N HCl (pH 1.2);
- Determine dissolution properties of capsuled mixtures in distilled water, phosphate buffer (PBS, pH 6.8) and 0.1 N HCl (pH 1.2) and;
- Perform thermal analysis (DSC) and x-ray powder diffractometry (XRPD) on various SP combinations.

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# CHAPTER 2

# MALARIA – AN INFECTIOUS DISEASE

## 2.1 Malaria

### 2.1.1 Introduction

Over the past 50 years, the incidence of malaria infections showed to decrease, but with 40% of the world's population living in endemic areas (Figure 2.1), the mortality and morbidity rate of malaria is still a major concern. In 2015, 214 million infections and 438 000 deaths were reported and according to the World Health Organisation (WHO), 88% of these deaths occur within the African region (WHO, 2015).

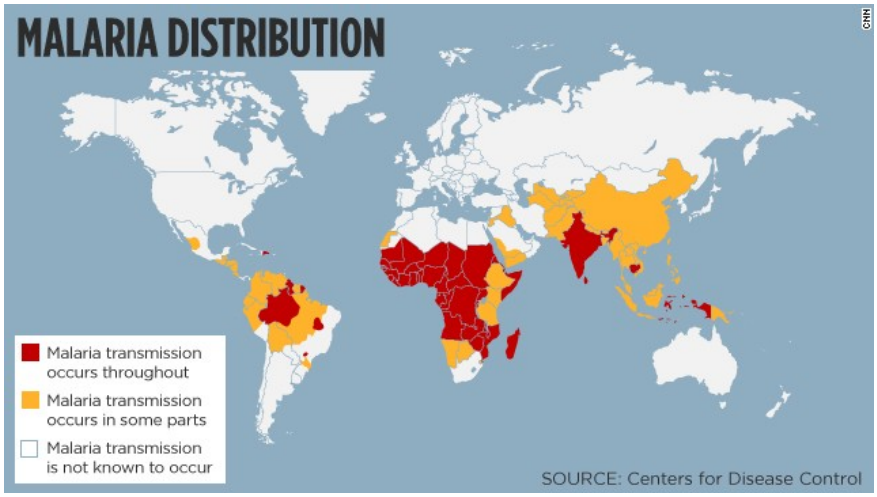


**Figure 2.1**: Malaria distribution across the world (Obtained from Centres of Disease Control and Prevention. 2012).

In the sub-Saharan region, children under 5 years, pregnant women and immuno-compromised individuals are at a high risk of becoming infected with malaria. At least one child living in Africa is killed by malaria every 30 seconds. Due to hormone changes, pregnant women are more likely to become infected with malaria than non-pregnant women. This is due to separation of erythrocytes in their 'possible' immune-compromised state and therefore the symptoms and outcomes during pregnancy are more severe which could lead to the death of the mother and her baby (Wells *et al.*, 2009:879).

### 2.1.2 History of malaria

Malaria, an ancient disease, although not fully understood, was documented by the Chinese, about 2700 years before Christ (BC). It also appeared on the clay tablets of Mesopotamia (2000 BC), in the papyri of the Egyptians (1570 BC) and in the Hindu texts (600 BC). The

Greeks, including Homer (850 BC), Empedocles of Agrigentrym (500 BC) and Hippocrates (400 BC) were aware of the poor health characteristics, fevers and enlarged spleen that occurred in the people that lived in marshy areas. For many years they believed malaria was caused by miasmas that rose from the swamps nearby but it wasn't until 1880 that scientists first started to understand malaria (Cox, 2010:1).

In 1880, scientific studies on malaria became possible after Charles Louis Alphonse Lavern, French military physician working in Algeria, discovered that parasites are the cause of the malaria disease. He studied numerous blood samples of the soldiers with a fever and noticed movable filaments (flagella) in the bloodstream. He claimed that these protozoa are responsible for the cause of malaria (Haas, 1999:520).

In 1898, Sir Ronald Ross, discovered that the protozoa are located in the mosquitoes' stomach walls and salivary glands which helped him to work out the life cycle of the Plasmodium parasite. He used a bird as a model and noted that the *Anopheles* female mosquito is the only vector that is responsible for the transmission of the parasite during a blood meal. (Cox, 2010:1; Haas, 1999:520; Symington, 2012:2).

### 2.1.3 Epidemiology

Malaria species are located in different regions around the world. *P. falciparum* is found in Papua New Guinea, Solomon Island and in the Sub-Saharan African areas whereas *P. ovale* is located in West Africa, *P. vivax* in the sub-continental regions of India and *P. malaria* in large parts of Africa. Transmission of malaria usually occurs in areas where the temperature is favourable and humans living side by side with the infected malaria mosquitoes (Pasvol, 2005:39).

"Airport malaria" is also a major concern when travellers visit endemic areas and return to non-endemic areas. The infected mosquito is transported in a travel bag or airplanes and they are responsible for infecting individuals in non-endemic areas. These infected individuals pass the infection on to uninfected mosquitoes, increasing the risk of individuals in non-endemic areas. It is therefore important to consider malaria in travellers with a fever and in patients after blood transfusions, needle stick injuries and organ transplantations (Pasvol, 2005:39).

### 2.1.4 Pathogenesis

The signs and symptoms a patient experience during malaria infection is caused by the parasite in its asexual form. The parasite invades and destroys the red blood cells (RBC) which is localized in the tissues and specific organs. Binding to the endothelial cells (cyto-adherence) it subsequently promotes the release of countless pro-inflammatory cytokines (tumour necrosis factor – TNF α) (Kwiatkowski *et al.*, 1989:364; Newton *et al.*, 1998:8).

The invasion of the mezoroites is ordered, specific, sequential and also the initiating step of the pathogenesis process. *Via* trophozoites, the ring of the *P.falciparum* parasite matures and forms the schizont stage. Inside the brain, the schizont-infected RBC binds themselves to the endothelial cells that are located in the post-capillary venules (Pasvol, 2005:39).

From the peripheral circulation, cyto-adherence is the responsible factor that causes the absence of the mature *P.falciparum* forms (sequestration) and due to sequestration of the parasite; micro-vascular obstruction occurs. Cyto-adherence is also localized and cytokine release is possible due to the endothelial cell activation / damage which are caused by the putative parasite “toxins” (Pasvol, 2005:39).

Mature parasites can “rosette”. This process involves the binding of the RBC that contains more mature stages, to the surface of uninfected RBC which causes micro-circularly obstruction. If the parasite rosette, it may be associated with severe malaria which can result in life-threatening complications (Pasvol, 2005:39).

### 2.1.5 Life cycle

The life cycle of the *falciparum* parasite (Figure 2.2) is a complex process as it requires an insect (mosquito) and a human host to go through all the different phases during the cycle (Biamonte *et al*, 2013:2829).

The exogenous sexual phase is the first phase during the life cycle and during this stage the female and male gametes combine in the middle gut of the mosquito. After the exogenous sexual phase the parasite starts to multiply in the gut (exogenous asexual stage - sporogony) and it is then followed by the multiplication of the parasite within the vertebrate host (endogenous asexual phase - schizogony). Near the end of the phase, the parasite starts to develop in the liver parenchymal cells (pre-erythrocyctic schizogony) and in the RBC (erythrocytic schizogony) (Garcia *et al.*, 2006:688).

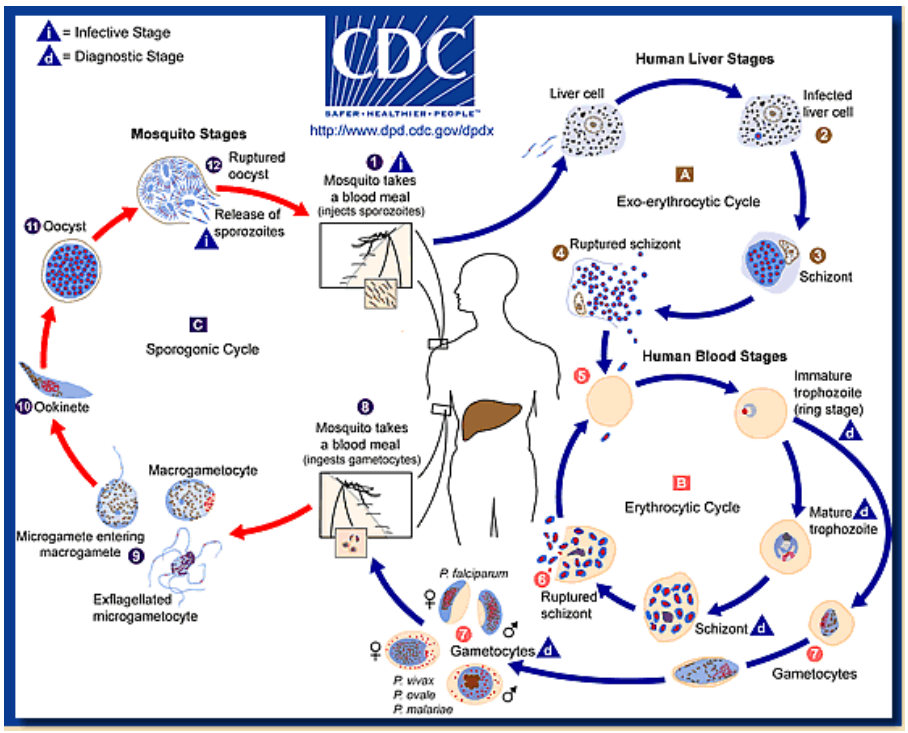


**Figure: 2.2:** The life cycle of the malaria parasite (Obtained from Centres of Disease Control and Prevention. 2012).

### 2.1.5.1 Exo-erythrocytic cycle

The exo-erythrocytic cycle starts when an infected *Anopheles* mosquito transmits the malaria parasite to the human host during a blood meal. The host is inoculated with sporozoites which has a larva-like morphology. Once these sporozoites reach the bloodstream, invasion of the liver occurs within 30 minutes (Biamonte *et al.*, 2013:2829). The sporozoites glide on the epithelial cells and bind themselves to the sinusoidal cells, crossing the Kuffer cells and migrating through the hepatocytes. Several hepatocytes are severely wounded during migration and reaching a viable cell, they invade the cell and the parasite start to multiply so that it creates thousands of new parasites (Leroy *et al.*, 2014:480).

### 2.5.1.2 Erythrocytic cycle

Reaching the culminate point of the phase (5 - 10 days), the vesicles that are filled with parasites, merosomes, burst and the erythrocytic infective parasites, merozoites, are released into the bloodstream (Leroy *et al*., 2014:480). The merozoites recognize, bind and invade the RBC and are located in the parasitophorous vacuole of the erythrocytes. The intra-erythrocytic development of the parasites starts to go through multiple forms (rings, trophozoite, schizont). Twenty (20) daughter merozoites are then formed and released into the bloodstream. They travel through the bloodstream to infect new RBC and a few of these merozoites develop male and female gametocytes. The gametocytes are transported to the gut of the female mosquito after a blood meal from the human host. The male gametes fuse with the female gametes and

form diploid ookinetes which migrate to the mid-gut, passing through the wall to form oocysts (Biamonte *et al.*, 2013:2829, Timothy *et al.*, 2009:881).

New sporozoites are then formed during meiotic division. These sporozoites travel and invade the salivary glands of the mosquito and the life cycle starts again after the infected mosquito inoculates a new human host with the malaria parasite (Biamonte *et al.*, 2013:2829, Timothy *et al.,* 2009:881).

### 2.1.6 Signs and symptoms of malaria

Malaria is categorised into uncomplicated (non-lethal) and severe malaria (life-threatening). Different clinical features are present during these infections but signs and symptoms such as fever, chills, dizziness, headaches, malaise, myalgia, abdominal pain, dry cough, diarrhoea, nausea and vomiting are common in most of the patients (Trampuz *et al.*, 2003:316).

#### 2.1.6.1 Uncomplicated malaria

Three stages are present during uncomplicated malaria. The cold stage consists of a cold sensation with shivering; the hot stage includes seizures in children, vomiting, headaches and fever. Finally a patient experience the sweating stage which consist of tiredness, sweats with a drop in temperature to normal. These attacks can last up to 6-10 hours (CDC, 2015).

#### 2.1.6.2 Severe malaria

Clinical features present during severe malaria include convulsions, impaired consciousness, prostration, circulatory collapse, acute injury of the kidneys, jaundice, vital organ dysfunction and abnormal bleeding, difficult breathing and respiratory distress. Syndromes such as acute pulmonary oedema and acute respiratory distress are also common. The systolic blood pressure in children is < 50 mm HG and < 80 mm HG in adults (WHO, 2012:7).

Complications such as severe anaemia, cerebral malaria, acute renal impairment, metabolic acidosis, shock, hypoglycaemia, pulmonary oedema, and bleeding may occur during severe malaria and could result in death within a few hours or days if the development is rapid and treatment is not started immediately (Trampuz *et al.*, 2003:316; WHO, 2012:43-54).

Patients older than 65 years, pregnant women, non-prophylaxis usage, severity of the illness and patients with an impaired immune system are at a greater risk of becoming infected with severe malaria. Children (1 month to 5 years of age) and travellers are also more susceptible for infection in tropical endemic areas. If a patient presents with any of these complications, they must be hospitalised and should immediately receive parenteral antimalarial chemotherapy (Trampuz *et al.*, 2003:316).

### 2.1.7 Diagnosis

Clinical features mentioned in 2.1.6 are important to consider in patients living in a malaria area or in travellers returning from a malaria area, regardless of their anti-malarial drug history. Several tests can also be done to diagnose malaria in these patients. These tests include:

- Thick and thin blood smear examination (light microscopy);
- Fluorescent microscopy;
- PCR based techniques;
- Antigen detection;
- Automated systems (Gkrania-Klotsas, 2007:79-80).

### 2.1.8 Prevention and Treatment

#### 2.1.8.1 Prevention

Bite prevention are crucial against malaria infection and also the first line of defence even during the usage of chemo-prophylactic drugs. Travellers travelling to and residents living in malaria infected areas need to consider the following bite prevention measures:

- Usage of insect repellents such as DEET (N,N-diethyl-m-toluamide) and picaridin;
- Permethrin and synthetic insecticides;
- Sleeping under insecticide-treated bed nets (in- and outdoors);
- Covering the arms and legs with long sleeve and loose clothing while wearing socks and shoes;
- Clothing can be treated, sprayed or impregnated with permethrin;
- Air conditioning and ceiling fans inside are useful to keep the room temperature cool;
- Fine mesh should be used to cover any entry route;
- Insecticides such as pyrethroid can be used to spray the room before dusk so that mosquitoes can be killed that entered the room during the day (Chiodini *et al.,* 2003:27-29).

#### 2.1.8.2 Antimalarial drugs

Antimalarial drugs are used as prophylaxis, treating falciparum and non-falciparum malaria. The chemo-prophylactic drugs eliminate the erythrocytic parasite before they can multiply to a certain level and therefore preventing clinical diseases (Saifi *et al.,* 2012:148).

The antimalarial drugs are divided into different classes. The drugs act as a) tissue schizonticides, b) blood schizonticides and c) gametocides. Tissue schizonticides are responsible for the elimination of dormant and developing liver forms; blood schizonticides target the erythrocytic parasites and gametocides preventing transmission to the parasite (Chamber & Deck, 2009:877).

### 2.1.9 Malaria in pregnancy

It is stated that the immuno-compromised state of pregnancy, hormonal changes and the separation of erythrocytes make pregnant women more susceptible for malaria infection than non-pregnant women. It is estimated that 25 million pregnant women who live in endemic areas are currently infected with malaria. More than 10 000 maternal deaths and 75 000 to 200 000 infant deaths occur in the Sub-Saharan area each year (Desai *et al.*, 2007:93; Schantz-Dunn *et al.*, 2009:189; McClure *et al.*, 2013:103).

In the late 1960's, published studies already described the adverse effects of malaria that a pregnant women may experience during infection. It is stated that low birth weight and maternal anaemia are two of the most common effects, but stillbirth and pre-term birth could also affect the mother and her baby (McClure *et al.*, 2012:103).

Controlling malaria in pregnant women could save the lives of the mother and their babies. Guidelines that may be followed by each individual to prevent the risk of mosquito bites and infection include:

1. As part of antenatal care, pregnant women must be provided with intermittent preventive therapy (IPTp) in their first or second pregnancy. The IPTp should consist of sulfadoxine (S) and pyrimethamine (P) and dosing should start in the second trimester. IPTp-SP must be given at regular intervals (at least 3 doses, 1 month apart). SP combination is safe, available worldwide, cheap and most pregnant women tolerate SP combination well (WHO, 2015:100).
2. Uncomplicated malaria must be managed as follows: Quinine in combination with clindamycin should be given in the first trimester for seven days (WHO, 2015: 48).

3. Pregnant women living in malaria areas should be provided with insecticide-treated nets (ITN). They should be encouraged to sleep under INT's for the whole duration of their pregnancy and also after delivery (WHO, 2015:102).

### 2.1.10 Resistance

The resistance to sulfadoxine and pyrimethamine continues to rise but are still used as IPTp in pregnant women in several countries in the sub-Saharan African region. In a recent study three or more doses of IPTp-SP (in a wide range of resistance to SP) were given to pregnant women. In comparison with the two dose regime, three or more doses during pregnancy resulted in a lower possibility of maternal malaria, higher birth weight and a lower risk of malaria anaemia in the first or second pregnancy (Kayentao *et al.,* 2013:595).

In certain parts of Africa, the *Plasmodium falciparum* parasite carries quintuple mutations which are responsible for the resistance to SP; but despite these mutations preventing adverse consequences (maternal and foetal outcomes) of malaria with IPTp-SP remains an effective choice. Based on the results, the WHO still recommends that all pregnant women who live in moderate-to-high transmission areas must be provided with IPTp-SP during every antenatal visit (WHO, 2015:102).

IPTp-SP plays an important role during malaria prevention in pregnant women; but the constant increase of the resistance to SP and the very little information regarding the physico-chemical characteristics, solubility and permeability properties of SP remains a major concern. More information of these properties could lead to possible modification of the drugs which may increase the therapeutic effectiveness of the IPTp-SP and thus decreasing the resistance to SP.

## 2.2 Sulfadoxine and pyrimethamine

### 2.2.1 Introduction

Sulfadoxine and pyrimethamine (SP) is a fixed-dose combination that contains 500 mg sulfadoxine and 25 mg pyrimethamine per tablet and both are classified as folate antagonists. These two drugs act together and inhibit the folate pathway. It then decreases the synthesis of pirimidine and reduces serine, DNA and methione formation (Saifi *et al.*, 2013:148).

### 2.2.2 Sulfadoxine

#### 2.2.2.1 Pharmacological classification and mechanism of action

Sulfadoxine (Figure 2.3), also known as sulfadoxinum, is a long-acting sulfonamide that inhibits dihydropteroate synthase (DHPS). This enzyme is responsible for utilizing *para*-aminobenzoic

acid (PABA) and therefore inhibits dihydropteroic acid synthesis. It is also a part of the folate metabolic pathway and DHFR (Dzinjalamala, 2004:3601).

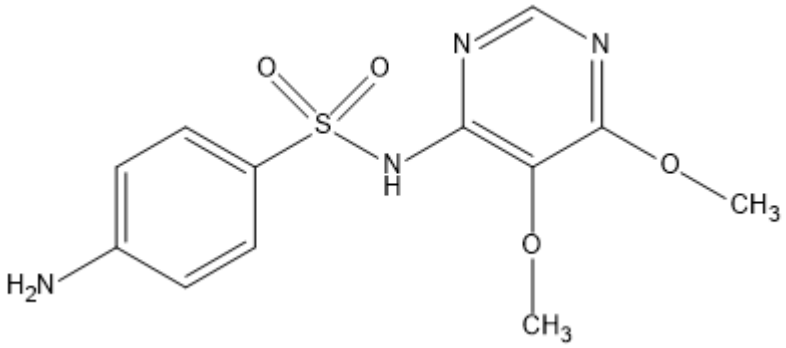


**Figure 2.3**: Chemical structure of sulfadoxine (BP, 2016).

#### 2.2.2.2 Pharmacokinetics

Sulfadoxine is absorbed from the gastrointestinal tract and after 4 h it reaches a plasma peak level of 60 mg/L. The volume of distribution is 2.3 L/kg, it binds to plasma proteins and the half-life time ranges between 100-230 hours. Glucuronidation is responsible for the metabolism of sulfadoxine and is being excreted unchanged in the urine (Gutman *et al.*, 2012:4).

#### 2.2.2.3 Physico-chemical properties

It is an odourless, crystalline powder which is white in appearance. It is poorly soluble in water and slightly soluble in ethanol and methanol. The chemical name of sulfadoxine is 4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide, with a molecular mass of 310.3 g/mol (Ph.Int., 2015:2). Sulfadoxine melts at 198°C with decomposition (BP, 2016).

### 2.2.3 Pyrimethamine

#### 2.2.3.1 Pharmacological classification and mechanism of action

Pyrimethamine or pyrimethaminum (Figure 2.4) is a dihydrofolate reductase inhibitor (DHFR) of plasmodia. Biosynthesis of purines and pyrimidines which is crucial for cell multiplication and DNA synthesis are blocked and leads to nuclear division throughout schizont formation in the liver and erythrocytes (Dzinjalamala; 2004:3601).

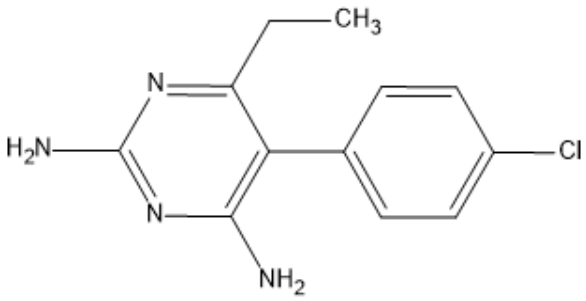


**Figure 2.4:** Chemical structure of pyrimethamine (BP, 2016).

#### 2.2.3.2 Pharmacokinetics

Pyrimethamine is absorbed from the gastrointestinal tract and after 4 h a plasma peak level of 0.2 mg/L is reached. Pyrimethamine binds to plasma proteins and its volume of distribution is 0.14 L/kg. The half-life of pyrimethamine is between 54 - 148 hours and during metabolism several unidentified metabolites are formed and excreted in the urine (Gutman *et al.*, 2012:4).

#### 2.2.3.3 Physico-chemical properties

It is an odourless, crystalline powder which is white in appearance, practically insoluble in water and partially soluble in ethanol and acetone. Its chemical name is 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine, with a melting range between 239 - 242°C and a molecular mass of 248.7 g/mol (Ph.Int., 2015).

### 2.2.4 Clinical uses of SP

SP combinations are used as a chemo-prophylactic drug against malaria. It is primarily used as intermittent preventive therapy in non-infected HIV pregnant women and is an effective combination against malaria when given in intervals (at least a month apart) two to three times during pregnancy (Gutman *et al.*, 2012:4).

### 2.2.5 Special precautions and side-effects of SP

#### 2.2.5.1 Special precautions

- Not recommended for neonates (< 8 weeks);
- Hypersensitivity to sulphonamides;
- Not recommended in the 1st trimester during pregnancy and 4 weeks before delivery;
- Severe renal and hepatic dysfunction (UNICEF, 2000:12).

#### 2.2.5.2 Side-effects

SP is normally well tolerated and side-effects may be present in 1 - 2% of individuals. These side-effects may include:

- Fatigue;
- Vomiting;
- Nausea;
- Skin irritations;
- Pruritus;
- Abdominal discomfort;
- Headaches (Gutman *et al.*, 2012:4; SAMF, 2010:507).

A few severe side-effects such as eosinophilia, leukopenia, haemolytic anaemia, trombocytopenia, megaloblastic anaemia, aplastic anaemia, and bone marrow suppression including agranulocytosis may be seen in only a few individuals (Gutman *et al.*, 2012:4).

### 2.2.6 Dissolution and solubility of SP

Therapeutic effectiveness of drugs can be related to the blood concentrations after drug administration. A number of properties such as solubility, the rate of dissolution, the permeability and metabolism of the drug or drug products influences oral bioavailability and need to be taken into consideration in order to obtain a therapeutic drug concentration. These properties contribute to the success or failure of drugs during clinical trials and it is therefore important to address and optimise these specific properties as much as possible (Jambhekar *et al.*, 2012:1174).

Solubility is defined by Patel *et al*. (2012:1459) as “the maximum amount of solute that dissolves in a certain quantity of a solution at a specific temperature.” It is known that more than 40% of oral dosage forms that are currently on the market show poor solubility in water which affects the dissolution rate, subsequently resulting in poor bioavailability. This may cause low therapeutic efficiency of the drug, therefore compromising drug prophylaxis and the possible exposure to sub-lethal drug concentrations (Jambhekar *et al.*, 2012:1174). Since sulfadoxine is poorly soluble in water (refer to 2.2.2.3) and pyrimethamine practically insoluble in water (refer to 2.2.3.3) drug resistance may be elevated contributing to the ineffective treatment of malaria.

Drug dissolution is defined by Peng *et al.* (2007:88) as a “process to which a solid substance dissolves in a specific medium.” It is an essential step in drug absorption for a drug to obtain acceptable therapeutic concentration so that a pharmacological response can be acquired.

Earlier studies indicated that variability in dissolution results exist for the SP products available on the market. Amin *et al.* (2005:3) collected thirteen SP samples from the Kenyan market for dissolution tests. Only three of the thirteen SP samples met the United States Pharmacopeia (USP) requirements (Q = 60% for both actives in 30 minutes). In another study, Amin and Kokwaro (2007:433) obtained fourteen SP samples from various African countries. Only four out of the fourteen SP samples met the USP requirements. In both studies pyrimethamine was indicated to be the cause of the poor results.

## 2.3 Conclusion

Although the resistance to sulfadoxine and pyrimethamine continues to rise, the WHO still recommends that pregnant women living in moderate-to-high transmission areas should receive IPTp-SP during each antenatal visit in the second and third trimester. Kayentao *et al*. (2013:595) indicated that three or more doses, during pregnancy, resulted in positive effects in a wide range of resistance to SP for the mother and baby and making it a crucial combination of drugs for pregnant women living in the sub-Saharan African region.

A major concern is the on-going mutations of the malaria parasite which result in resistance to SP. The mentioned problem of very poor solubility and slow dissolution rates of SP must be evaluated and considered critically. These two very important properties of the two drugs might actually trigger the development of parasitic resistance. Therefore, re-thinking the way that SP combinations are used and formulated could provide a positive step towards combating the parasitic infection and counter the development of resistance. Very little information is however available regarding the properties of these two drugs. It is therefore important to investigate and obtain more information regarding these properties so that SP can be modified and formulated in such a way that there is no further increase in the development of resistance of the malaria parasite to SP and to maintain the therapeutic effectiveness of these two drugs.

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# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Introduction

During this study the main focus was the determination of solubility and dissolution rates of sulfadoxine and pyrimethamine as single compounds and in different combination ratios. Analytical techniques employed during solubility and dissolution studies and the determination of certain physico-chemical characteristics are presented in this chapter.

## 3.2 Materials

Sulfadoxine and pyrimethamine were purchased from DB Fine Chemicals, Johannesburg, South Africa. All chemicals and reagents were at least of analytical grade. Ultrapure water with a resistivity of at least 18.1 megaohm was obtained from various water purification systems available in-house.

## 3.3 Study design

The different combination ratios of SP and the tests performed on these ratios are presented in Table 3.1. These combinations were used throughout the study to investigate the effect that the different ratios have on the solubility and dissolution behaviour of the two drugs. The two single compounds were included in all tests for comparison purposes.

**Table 3.1** Tests performed on various SP ratios

| Sulfadoxine (%) | Pyrimethamine (%) | Tests |
|---|---|---|
| 70 | 30 | Sol, Diss, DSC, XRPD |
| 65 | 35 | Sol, Diss |
| 60 | 40 | Sol, Diss |
| 55 | 45 | Sol, Diss, DSC, XRPD |
| 50 | 50 | Sol, Diss, DSC, XRPD |
| 40 | 60 | Sol, Diss |
| 30 | 70 | Sol, Diss, DSC, XRPD |
| 500 | 25 | Sol, Diss |
| **Sulfadoxine single component** | | Sol, Diss, DSC, XRPD |
| **Pyrimethamine single component** | | Sol, Diss, DSC, XRPD |

***Sol:** *Solubility*; **Diss:** *Dissolution*; **DSC:** *differential scanning calorimetry*; **XRPD:** *x-ray powder diffraction*

## 3.4 Methods

### 3.4.1 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a thermal analytical technique which is used to determine detailed information of different kinds of substances regarding their physical and energetic properties. Quantitative information such as endothermic, exothermic and heat capacity changes as a function of time and temperature are provided by the DSC. This includes melting, purity and glass transition temperature (Clas *et al*., 1999:311).

To record the DSC thermograms a DSC-60 Shimadzu instrument (Shimadzu, Kyoto, Japan) was used. Samples weighing approximately 3 - 6 mg were placed in aluminium crimp cells with pierced lids and heated to the desired temperature (maximum 300°C). The heating rate was set to 10°C/min, with 35 ml/min nitrogen gas purge. The single compounds and SP combination ratios' melting point were determined (Table 3.1).

### 3.4.2 X-Ray Powder Diffraction (XRPD)

By using XRPD the characterizing of crystalline or amorphous materials, quantitative analysis and identification of different phases can be done (Louër, 1999:2253). In this study, the XRPD was used to determine the crystalline structure of SP in different ratios (Table 3.1). Determining the crystalline nature/habit of a single drug or combinations of drugs is an important step during

research as it helps to determine the physical and chemical properties that will have an effect on the solubility and dissolution rate of drugs (Datta *et al*., 2004:42).

During the XRPD analyses in this study, samples were evenly distributed on a zero background sample holder. XRPD patterns were obtained using a PANalytical Empyrean diffractometer (PANalytical, Almelo, Netherlands). The XRPD measurement parameters are described in Table 3.2.

**Table 3.2:** XRPD measurement parameters

| | |
|---|---|
| **Target** | Cu |
| **Voltage** | 40 kV |
| **Current** | 45 mA |
| **Divergence slit** | 2 mm |
| **Anti-scatter slit** | 0.6 mm |
| **Detector slit** | 0.2 mm |
| **Scanning speed** | 2° /min |
| **Step size** | 0.025° |
| **Step time** | 1.0 sec |

### 3.4.3 High Performance Liquid Chromatography (HPLC)

The analysis for this study was performed according to the monograph for sulfadoxine and pyrimethamine FDC (fixed dose combination) as published in The International Pharmacopoeia (Ph.Int, 2016). The method was verified in-house by means of the following parameters: linearity, repeatability and range.

The following setups were used during the study:

**Analytical instrument 1:** Hitachi Chromaster (Tokyo, Japan) chromatographic system. The system consisted of a 5410 UV detector, an auto-sampler (5260) with a sample temperature controller and a solvent delivery module (5160).

**Analytical instrument 2:** Shimadzu (Kyoto, Japan) UFLC chromatographic system. The system consisted of a UV/VIS Photodiode Array detector (SPD-

M20A), a SIL-20AC auto-sampler with a sample temperature controller and a LC-20AD solvent delivery module.

**Column 1:** LC Column 250 mm x 4.6 mm, $C_{18,}$ 5 µm (Phenomenex Luna).

**Column 2:** Kinetex Core-Shell Technology, 250 mm x 4.6 mm, $C_{18,}$ 5 µm (Phenomenex Luna).

**Mobile phase:** To a 2000 ml volumetric flask 20 ml of 100% acetic acid glacial (GAA) and 1 ml Triethylamine (TEA) were accurately pipetted, followed by the addition of approximately 1600 ml of water. The pH was adjusted to 4.2 with 10 N NaOH. The solution was made to volume with HPLC water. Subsequently, 1600 ml of the prepared solution and 400 ml of acetonitrile was thoroughly mixed. The resulting mobile phase was degassed and filtered prior to use.

**Injection volume:** Depending on the type of sample, 5-20 µl was used.

**Temperature:** Ambient (20 – 25°C).

**Flow rate:** 2 ml/min.

**Detection wavelength:** 254 nm.

### 3.4.3.1 Preparation of standard stock solutions

#### 3.4.3.2 Sulfadoxine

Approximately 150 mg of sulfadoxine were weighed and transferred to a 50 ml volumetric flask. Subsequently, 20 ml of acetonitrile were then added; sonicated for 20 min and left to cool down. The solution was diluted to volume with mobile phase and the resulting mixture yielded a concentration of 3000 µg/ml.

Five different solutions were prepared to obtain a specific analytical value (Table 3.3). Mobile phase was used as diluent. These standards were analysed by means of the various instrument configurations as indicated in section 3.4.3. Linear regression analysis was performed on the obtained data.

**Table 3.3:** Sulfadoxine standard solutions for linearity analysis

| | Volume stock solution (ml) | Volume (ml) | Concentration (µg/ml) |
|---|---|---|---|
| STD 1 | 1 from STD 2 | 20 | 146 |
| STD 2 | 5 | 20 | 728 |
| STD 3 | 10 | 20 | 1455 |
| STD 4 | 13 | 20 | 1892 |
| STD 5 | 20 | 20 | 2910 |

**STD** – Standard solution

### 3.4.3.3 Pyrimethamine

Approximately 300 mg of pyrimethamine were weighed and transferred to a 100 ml volumetric flask. Acetonitrile (35 ml) was then added; sonicated for 20 min and left to cool down. The solution was diluted to volume with mobile phase and the mixture yielded a concentration of 3000 µg/ml.

Five different solutions were prepared to obtain a specific analytical value (Table 3.4). Mobile phase was used as diluent. These standards were analysed by means of the various instrument configurations as indicated in section 3.4.3. Linear regression analysis was performed on the data.

**Table 3.4:** Pyrimethamine standard solutions for linearity analysis

| | Volume stock solution (ml) | Volume (ml) | Concentration (µg/ml) |
|---|---|---|---|
| STD 1 | 1 from STD 2 | 20 | 7.49 |
| STD 2 | 5 | 20 | 150 |
| STD 3 | 10 | 20 | 749 |
| STD 4 | 13 | 20 | 2395 |
| STD 5 | 20 | 20 | 2994 |

**STD –** Standard solution

### 3.4.3.4 Preparation of PBS (pH 6.8)

Approximately 0.896 gram of sodium hydroxide pellets (NaOH) and 6.805 gram of potassium dihydrogen phosphate ($KH_2PO_4$) were quantitatively transferred to a 1000 ml volumetric flask. About 700 ml of ultrapure water was added and stirred until dissolved. The pH was measured and adjusted with either 1 N HCl or 1 N NaOH, as required. The resulting solution was subsequently diluted to volume with ultrapure water.

### 3.4.3.5 Preparation of 0.1 N HCl

Approximately 8.33 ml of concentrated hydrochloric acid (32%) was measured in a measuring cylinder and transfer to a 1000 ml volumetric flask containing about 700 ml of ultrapure water. The solution was stirred to ensure completed mixing followed by dilution thereof to volume with ultrapure water.

### 3.4.4 Solubility

Six samples of each single compound and of the various combination ratios (Table 3.1) were individually weighed. Each sample was placed into a test tube and 10 ml of solvent was pipetted into each test tube. Solvents were chosen to cover a wide pH range. Parafilm® (Neenah, USA), was used to seal the test tubes and a screw cap was tightly fitted onto every test tube to prevent leakage. Each test tube was then affixed to a rotating axis in a water bath. Subsequently the test tubes were rotated for a period of 24 h. After the sample agitation period the rotating axis was switched off and the samples were left for five minutes to settle. From each test tube a sample was collected, filtered and the necessary dilutions were made, and assayed by HPLC (See section 3.2.3).

The following parameters were used during the solubility studies:

| | |
|---|---|
| **Analytical instrument:** | Water bath |
| **Solvents:** | Distilled water |
| | PBS pH 6.8 |
| | 0.1 N HCl |
| **Temperature:** | 37 ± 2°C |
| **Run time:** | 24 hours |
| **Rotation speed:** | Axis rotating at 54 rpm |

### 3.4.5 Dissolution

Dissolution studies were performed on all combination ratios and the two single compounds as set out in Table 3.1. Sulfadoxine, pyrimethamine and different combination ratios of SP were weighed into gelatine capsules (size 1) (Capsugel, Johannesburg, South Africa). For each single compound or combination, six capsules were used and hand filled totalling a weight of 150 mg. Solvents were chosen to cover a wide pH range

During the dissolution study, each capsule was dropped into a vessel filled with the selected dissolution medium. Where necessary, sinkers were used to prevent the capsules from floating. After intervals of 10, 20, 30, 45 and 60 min, 5 ml of the solution were withdrawn from each vessel through a 0.45 µm filter and sampled into a test tube. After each withdrawal, 5 ml of of the selected medium (preheated to 37 ± 0.5°C) was replaced into each vessel. Where necessary, dilutions were made and the samples were analysed using HPLC (See section 3.2.3).

The following parameters were used during the dissolution studies:

| | |
|---|---|
| **Analytical instrument 1:** | LABINDIA (Thane, India) Dissolution Bath (Apparatus 2 – Paddles) |
| **Analytical instrument 2:** | VanKel700 Dissolution Bath (Cary, USA) (Apparatus 2 – Paddles) |
| **Dissolution media:** | Distilled water;<br>PBS pH 6.8;<br>0.1 N HCl |
| **Rotation speed:** | 75 rpm |
| **Temperature:** | 37 ± 0.5°C |
| **Withdrawal times (min):** | 10, 20, 30, 45, and 60 |

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Ph.Int ***see*** International Pharmacopoeia.

# CHAPTER 4

# RESULTS AND DISCUSSIONS

## 4.1 Introduction

As mentioned in the aims and objectives section (chapter 1), previous studies conducted on sulfadoxine and pyrimethamine combinations, showed improved solubility behaviour of both drugs. It was therefore imperative to investigate this behaviour further and to determine to what extent different sulfadoxine and pyrimethamine ratios influence the solubility of one another. Since solubility is a drug property that directly relates to both physical and chemical characteristics it was therefore imperative to investigate the improved solubility phenomenon *via* multiple analytical techniques. Results obtained during the complete study from all the tests discussed in chapter 3, were documented and are presented in this chapter.

## 4.2 Verification of validity of HPLC method

### 4.2.1 Results

Verification was performed on both the analytical instruments as previously described in paragraph 3.2.3. This was done by means of linearity, range and repeatability. Analytical instrument 1 was used as the primary instrument. Ranges covered on both instruments are displayed in Tables 4.1 – 4.4. Where results were obtained outside the linear range, the injection volume was adapted to deliver results within the linear range. For instrument 2, the concentration of standard five was too high and results therefore were limited to a smaller range.

The linear regression curves for both instruments are presented in Figures 4.1 – 4.4. Repeatability was evaluated on both instruments by injecting all standard solutions five times. Results are presented as a %RSD for each concentration level in Table 4.1 – 4.5.

### *Analytical instrument 1*

**Table 4.1** Results for linearity study of sulfadoxine on Instrument 1

| Concentration (µg/ml) | Average Peak Areas | %RSD |
|---|---|---|
| 145.50 | 6288985 | 0.45 |
| 727.50 | 30203550 | 0.18 |
| 1455.00 | 58817417 | 0.16 |
| 1891.50 | 81029622 | 0.49 |
| 2910.00 | 119785594 | 0.39 |

**Table 4.2** Results for linearity study of pyrimethamine on Instrument 1

| Concentration (µg/ml) | Average Peak Areas | %RSD |
|---|---|---|
| 7.49 | 335867 | 0.57 |
| 149.70 | 4080280 | 0.26 |
| 748.50 | 20929243 | 3.39 |
| 2395.00 | 65210315 | 0.13 |
| 2994.00 | 82357390 | 2.81 |

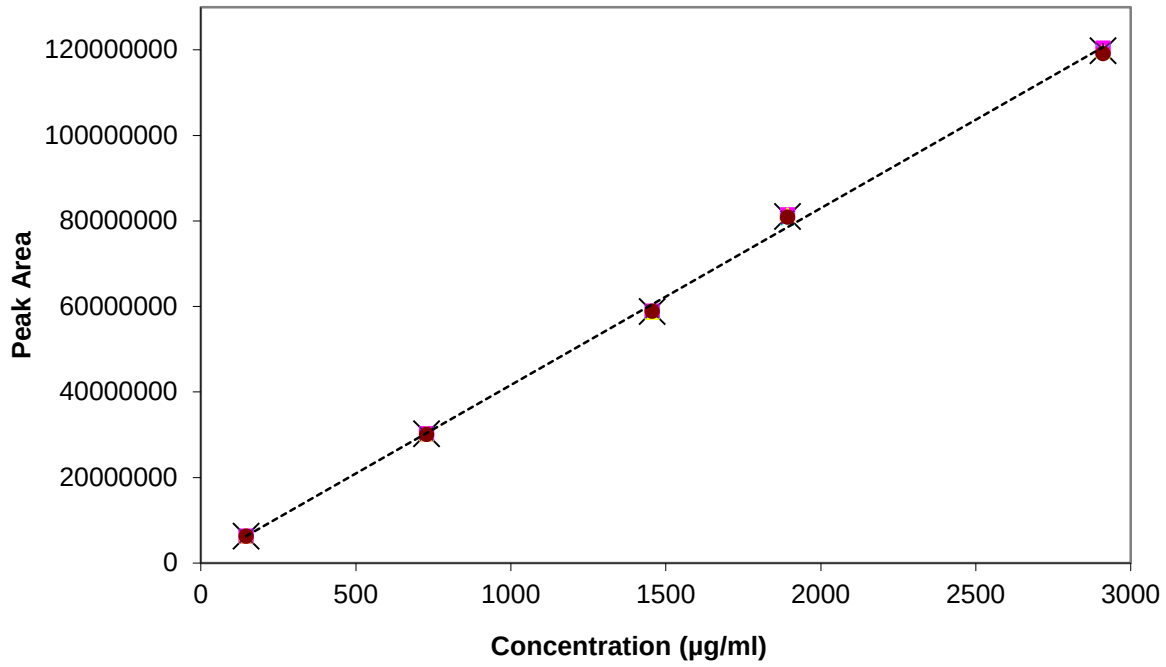


**Figure 4.1:** Linear regression graph of sulfadoxine on Instrument 1.

An $r^2$ – value of 0.9987 was obtained in the concentration range 145.50 – 2910 µg/ml. The equation for this line was calculated to be:

$$y = 41357x + 253889 \qquad (1)$$

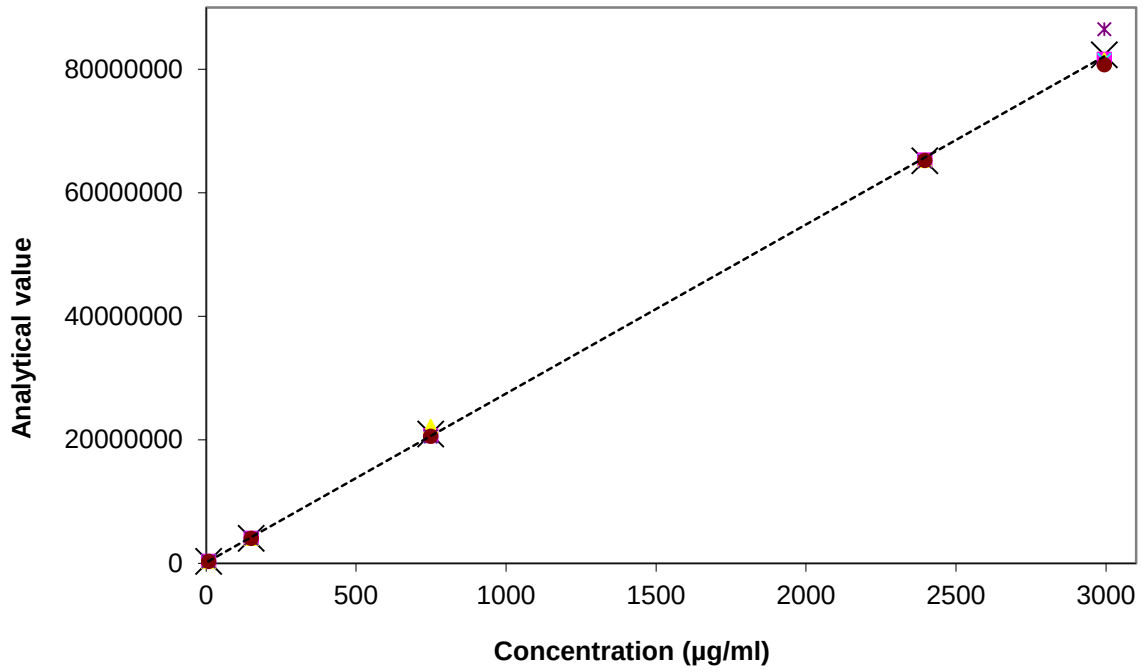


**Figure 4.2:** Linear regression graph of pyrimethamine on Instrument 1.

An $r^2$ – value of 0.9999 was obtained between concentrations 7.49 – 2994 µg/ml. The equation for this line was calculated to be:

$$y = 27364x + 133065 \quad (2)$$

### *Analytical instrument 2*

**Table 4.3:** Results for linearity study of sulfadoxine on Instrument 2

| Concentration (µg/ml) | Average Peak Areas | %RSD |
|---|---|---|
| 145.50 | 6446344 | 0.29 |
| 727.50 | 31382584 | 0.09 |
| 1455.00 | 63300258 | 0.32 |
| 1891.50 | 90814768 | 0.41 |

**Table 4.4:** Results for linearity study of pyrimethamine on Instrument 2

| Concentration (µg/ml) | Average Peak Areas | % RSD |
|---|---|---|
| 7.49 | 360676 | 3.86 |
| 149.70 | 4087618 | 0.27 |
| 748.50 | 20391910 | 0.30 |
| 2395.00 | 66452546 | 4.81 |

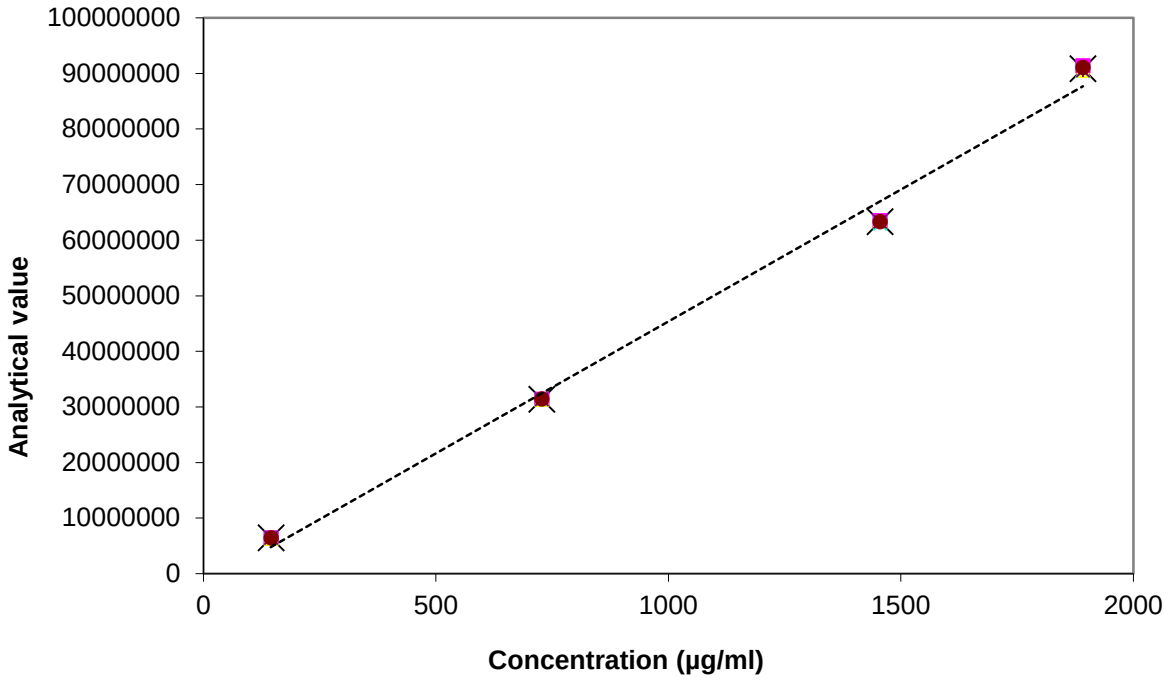


**Figure 4.3:** Linear regression graph of sulfadoxine on Instrument 2.

An $r^2$-value of 0.9934 was obtained in the concentration range 145.50 – 1891.50 µg/ml. The equation for this line was calculated to be:

$$y = 47470x - 2089069 \quad (3)$$

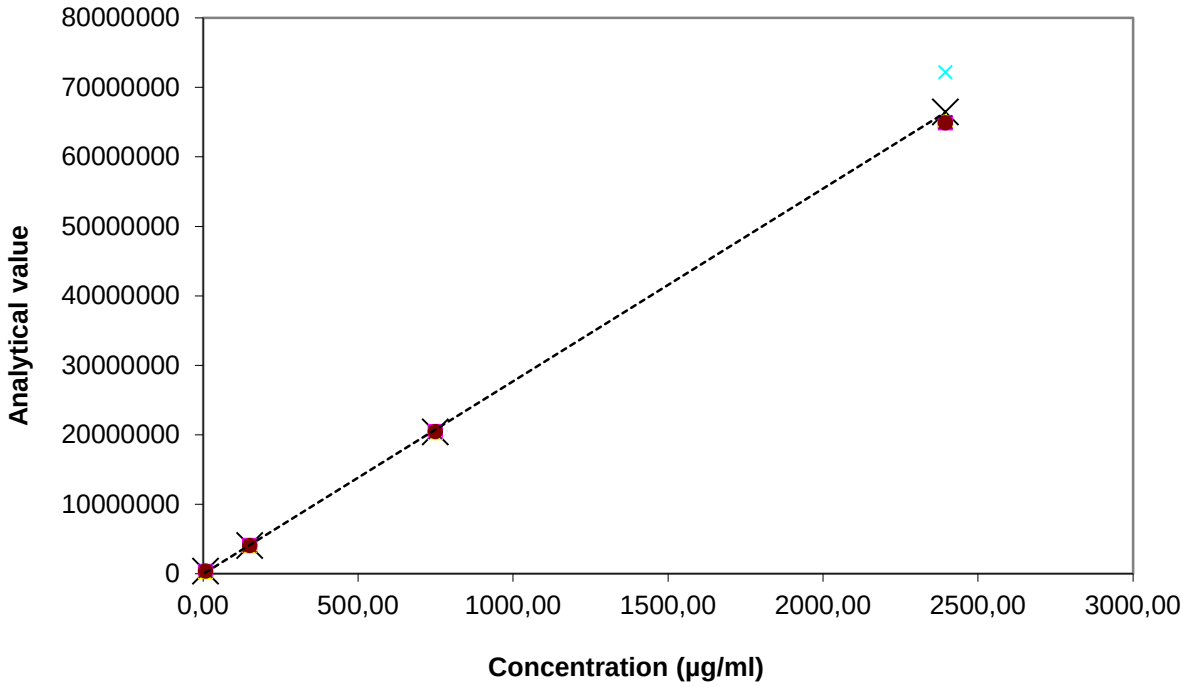


**Figure 4.4** Linear regression graph of pyrimethamine on Instrument 2.

An $r^2$-value of 0.9999 was obtained between concentrations 7.49 – 2395 µg/ml. The equation for this line was calculated to be:

$$y = 27732x - 60458 \qquad (4)$$

The results for STD 5 are omitted due to the fact that the instrument could not integrate the peaks as they were too large.

### 4.2.2 System suitability

System suitability was performed before and after each solubility and dissolution study by injecting a standard solution six times. The purpose was to verify the resolution between the active peaks (resolution > 2) and repeatability of the system (%RSD < 2.0 for six injections). No data was used unless these parameters were in compliance.

### 4.2.3 Discussion

A summary of the linearity and range results are indicated in Table 4.5.

**Table 4.5** Summarized validation results obtained for sulfadoxine and pyrimethamine on two different HPLC systems

| **Parameters** | **Analytical instrument 1** | | **Analytical instrument 2** | |
|---|---|---|---|---|
| | **Sulfadoxine** | **Pyrimethamine** | **Sulfadoxine** | **Pyrimethamine** |
| Linearity ($r^2$) | 0.998 | 0.999 | 0.993 | 0.999 |
| Range (µg/ml) | 145.50 - 2910 | 7.49 - 2994 | 145.50 – 1891.50 | 7.49 - 2395 |
| Repeatability | All %RSDs < 0.5 | All %RSD < 3.5 | All %RSD < 0.5 | All %RSD < 5 |

In all cases linearity was proven over the analytical ranges for sulfadoxine and pyrimethamine as all $r^2$ - values were larger than 0.99. The ranges were chosen to effectively accommodate all results obtained from the solubility and dissolution studies. The repeatability results indicated that in all cases a %RSD of less than 5% was obtained.

### 4.2.4 Conclusion

The method was verified to be acceptable for use on both analytical instruments.

## 4.3 Solubility

Solubility tests were done on the single components and the different ratio (%w/w) combinations as indicated in Table 3.1. These tests were done in distilled water, PBS (pH 6.8) and 0.1 N HCl (pH 1.2).

Results of the solubility tests done in distilled water, PBS and 0.1 N HCl are presented in Tables 4.6 – 4.14.

**Table 4.6:** Determined solubility concentration (*µg/ml*) of sulfadoxine single component

| **Medium** | ***Average Concentration (µg/ml)*** | **%RSD** |
|---|---|---|
| Distilled water | 244 | **3.39** |
| PBS | 1018 | **3.36** |
| 0.1 NHCl | 1190 | **0.71** |

**Table 4.7:** Determined solubility concentration (*µg/ml*) of pyrimethamine single component

| **Medium** | ***Average Concentration (µg/ml)*** | **%RSD** |
|---|---|---|
| Distilled water | 34 | **27.71** |
| PBS | 81 | **1.62** |
| 0.1 NHCl | 2714 | **5.21** |

**Table 4.8:** Determined solubility concentration (*µg/ml*) of the S500:P25 combination

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 464 | **2.98** | 280 | **0.68** |
| **PBS** | 1126 | **1.76** | 103 | **4.67** |
| **0.1 N HCl** | 1192 | **1.22** | 972 | **2.94** |

**Table 4.9:** Determined solubility concentration (*µg/ml*) of the S70:P30 combination

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 494 | **0.69** | 281 | **1.36** |
| **PBS** | 1044 | **2.62** | 101 | **9.36** |
| **0.1 N HCl** | 997 | **0.56** | 1173 | **18.10** |

**Table 4.10:** Determined solubility concentration (*µg/ml*) of the S60:P40 combination

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 490 | **1.16** | 249 | **1.52** |
| **PBS** | 1127 | **1.20** | 102 | **6.22** |
| **0.1 N HCl** | 948 | **0.86** | 1395 | **31.28** |

**Table 4.11:** Determined solubility concentration (*µg/ml*) of the S55:P45 combination

| | **Sulfadoxine** | | **Pyrimethamine** | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 496 | **0.76** | 247 | **1.25** |
| **PBS** | 1108 | **2.92** | 99 | **12.23** |
| **0.1 N HCl** | 953 | **0.88** | 1189 | **11.18** |

**Table 4.12:** Determined solubility concentration (*µg/ml*) of the S50:P50 combination

| | **Sulfadoxine** | | **Pyrimethamine** | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 503 | **3.79** | 252 | **3.44** |
| **PBS** | 1032 | **5.24** | 100 | **9.60** |
| **0.1 N HCl** | 916 | **1.50** | 1192 | **5.60** |

**Table 4.13:** Determined solubility concentration (*µg/ml*) of the S40:P60 combination

| | **Sulfadoxine** | | **Pyrimethamine** | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 477 | **3.03** | 254 | **5.55** |
| **PBS** | 965 | **16.31** | 106 | **16.31** |
| **0.1 N HCl** | 857 | **1.34** | 1917 | **2.27** |

**Table 4.14:** Determined solubility concentration (*µg/ml*) of the S30:P70 combination

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Medium** | **Average Concentration µg/ml** | **%RSD** | **Average Concentration µg/ml** | **%RSD** |
| **Water** | 493 | **0.40** | 283 | **1.02** |
| **PBS** | 1154 | **0.96** | 99 | **7.79** |
| **0.1 N HCl** | 812 | **1.66** | 1478 | **38.49** |

Figures 4.5 – 4.13 depict the final solubility concentrations for sulfadoxine, pyrimethamine and SP combinations in the three different media respectively. Comparative results for sulfadoxine, pyrimethamine and SP combinations in the respective media are presented in Figures 4.14 – 4.19.

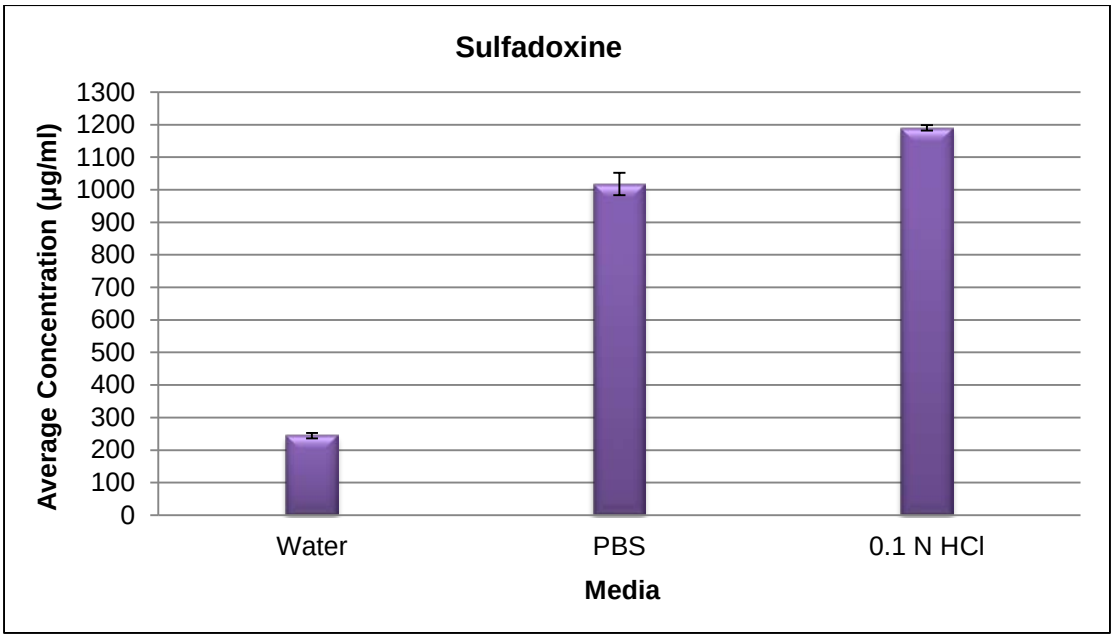


**Figure 4.5:** Determined solubility concentrations (*µg/ml*) of sulfadoxine single component in distilled water, PBS and 0.1 N HCl.

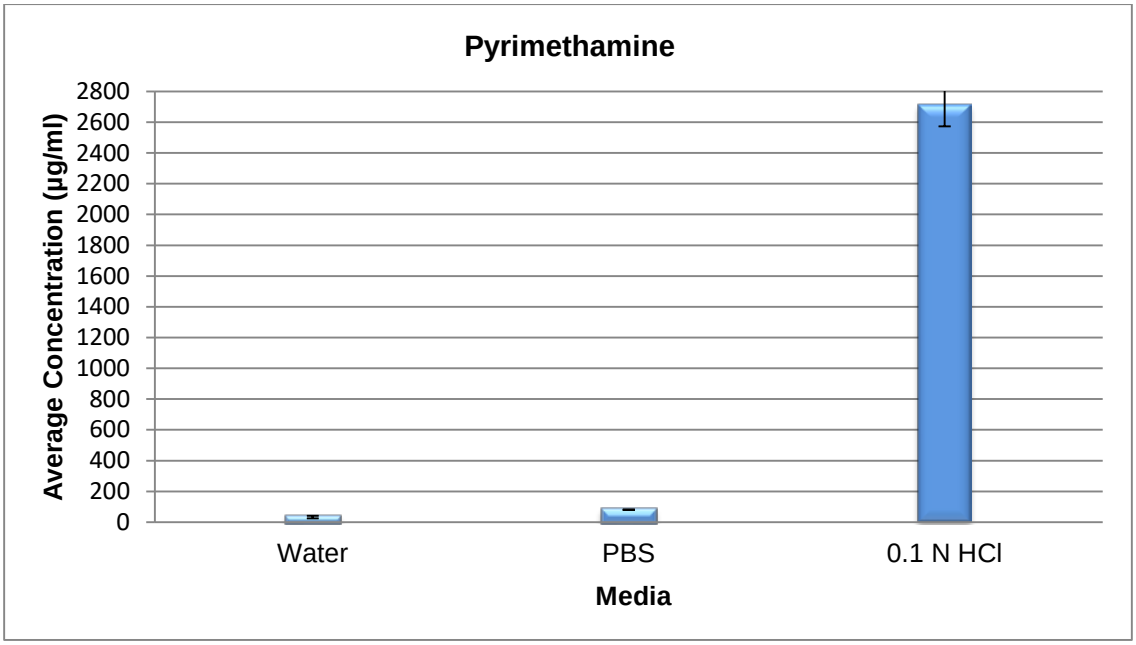


**Figure 4.6:** Graph depicting the determined solubility concentrations (*µg/ml*) of pyrimethamine single component in distilled water, PBS and 0.1 N HCl.

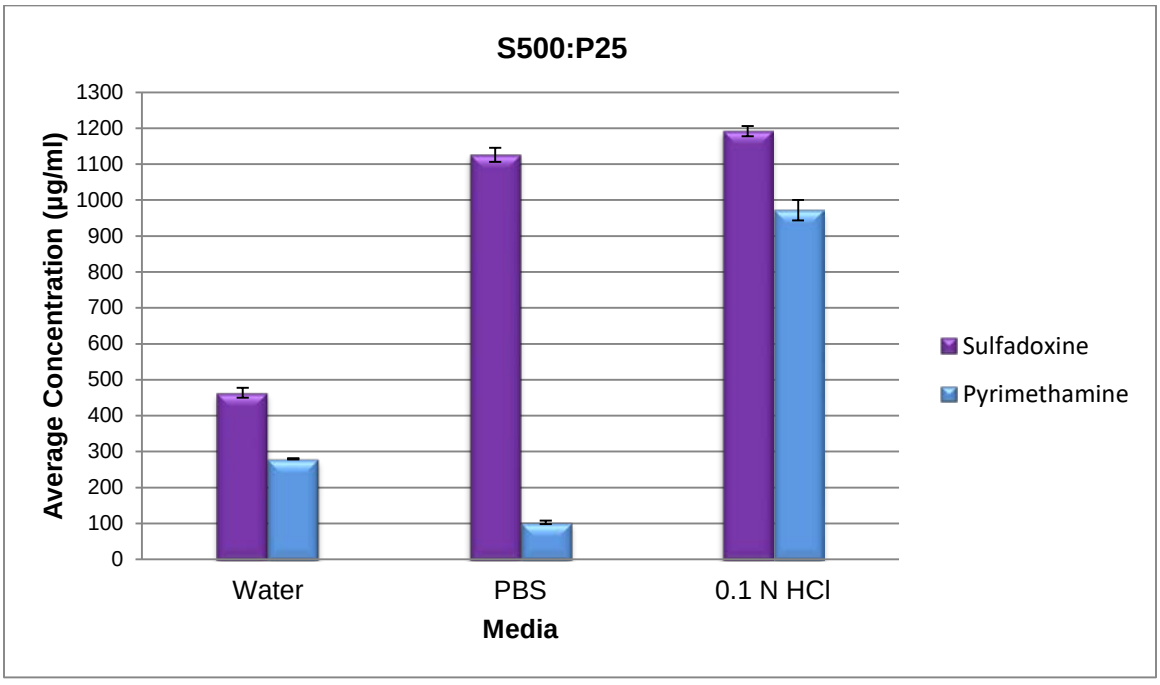


**Figure 4.7:** A combined graph of sulfadoxine and pyrimethamine concentrations (*µg/ml*) when in a S500:P25 (%w/w) combination in distilled water, PBS and 0.1 N HCl.

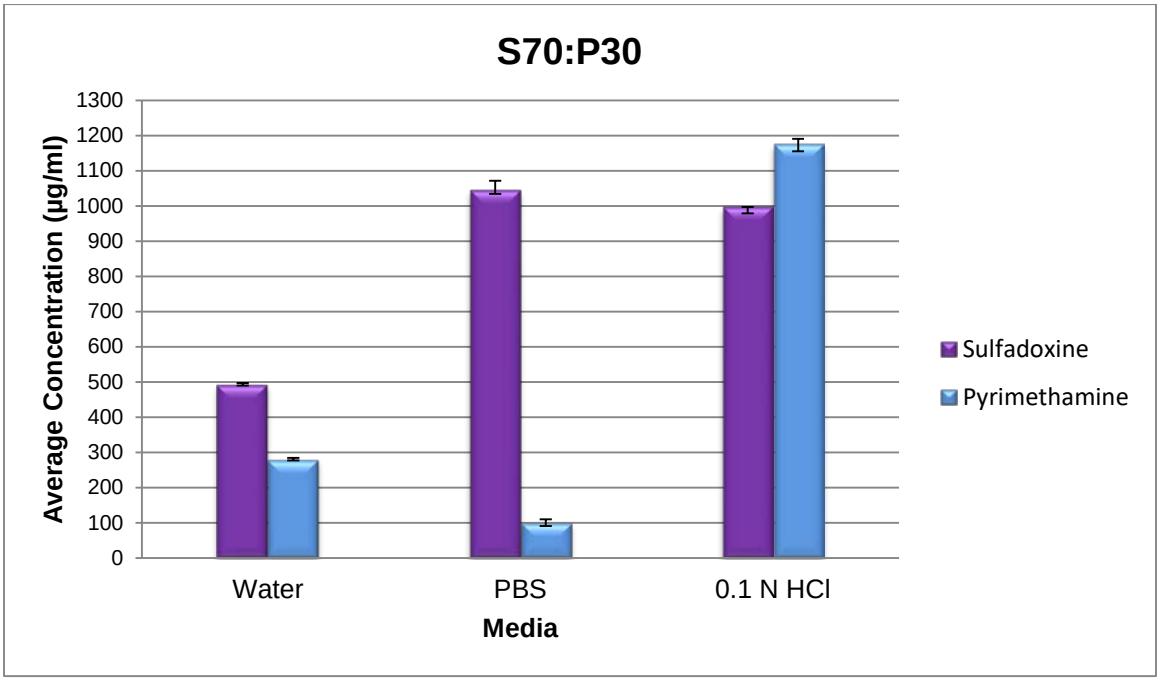


**Figure 4.8:** A combined graph of sulfadoxine and pyrimethamine concentrations (*µg/ml*) when in a S70:P30 combination in distilled water, PBS and 0.1 N HCl.

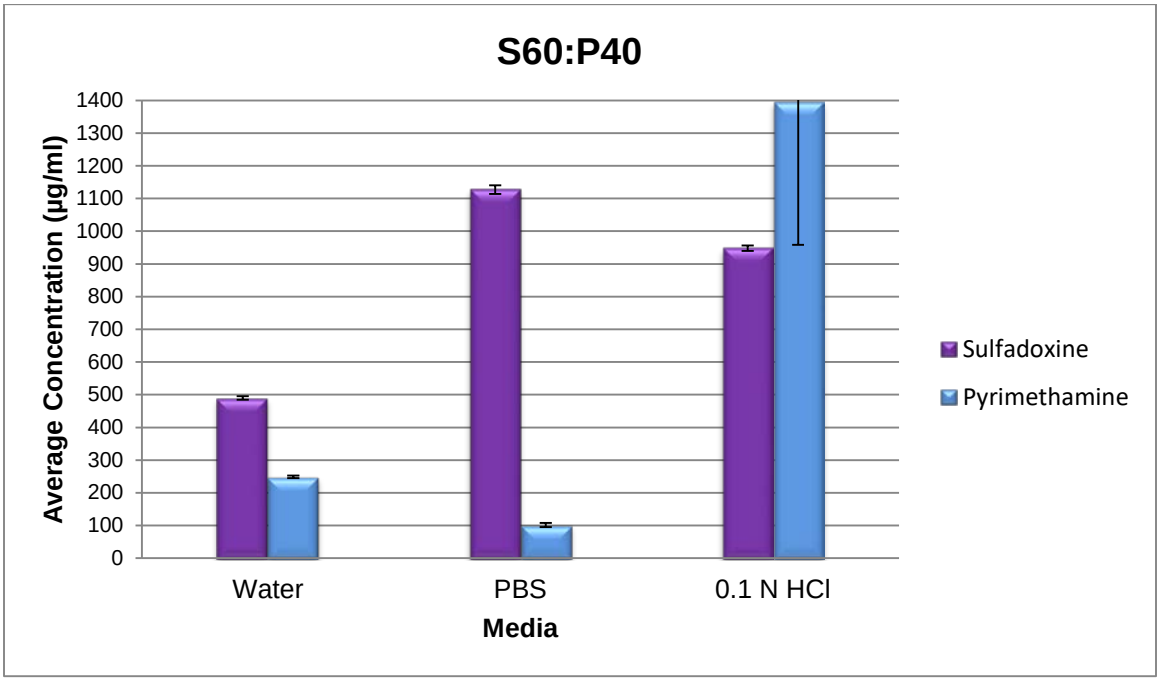


**Figure 4.9:** Graph depicting the solubility concentrations (*µg/ml*) of sulfadoxine and pyrimethamine determined from a %w/w ratio of combination S60:P40 in distilled water, PBS and 0.1 N HCl.

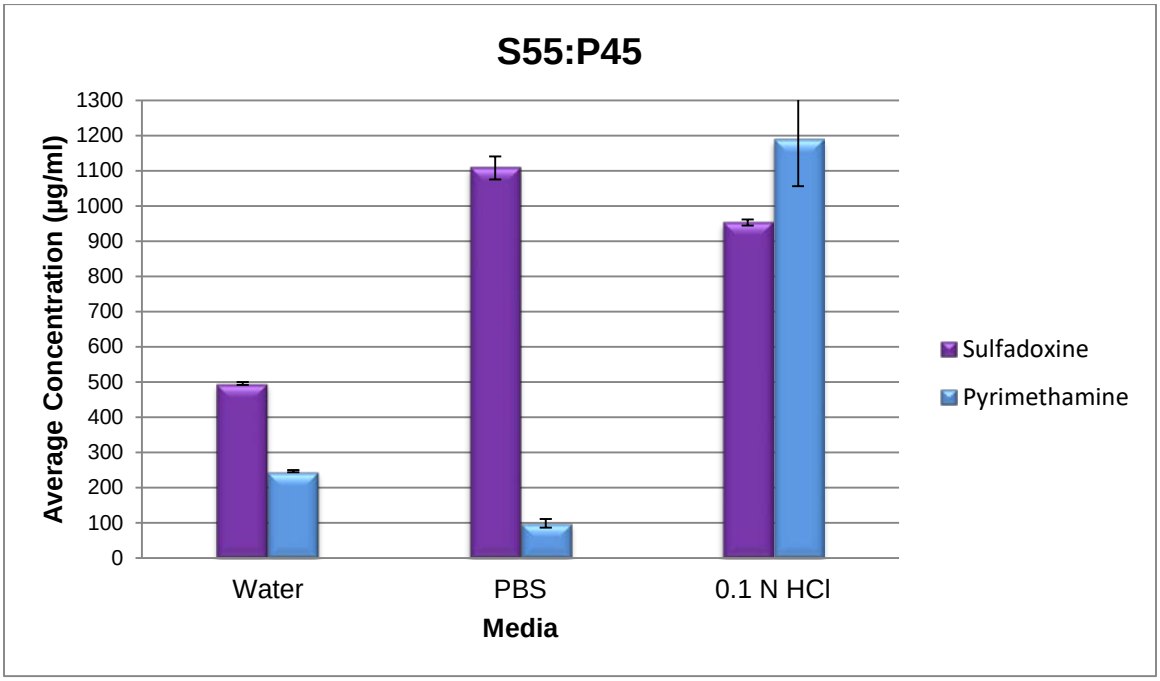


**Figure 4.10:** Graph showing the solubility concentrations (*µg/ml*) of sulfadoxine and pyrimethamine determined from a %w/w ratio of S55:P45 combination in distilled water, PBS and 0.1 N HCl.

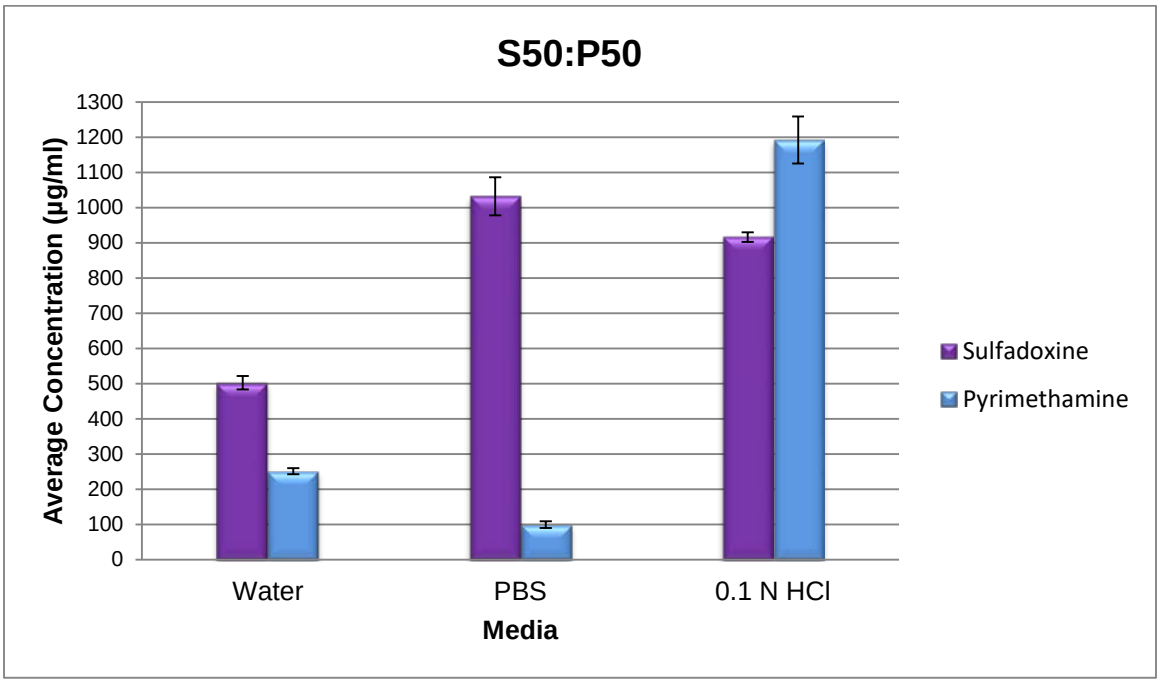


**Figure 4.11:** A combined graph of sulfadoxine and pyrimethamine concentrations (*µg/ml*) when in a S50:P50 combination in distilled water, PBS and 0.1 N HCl.

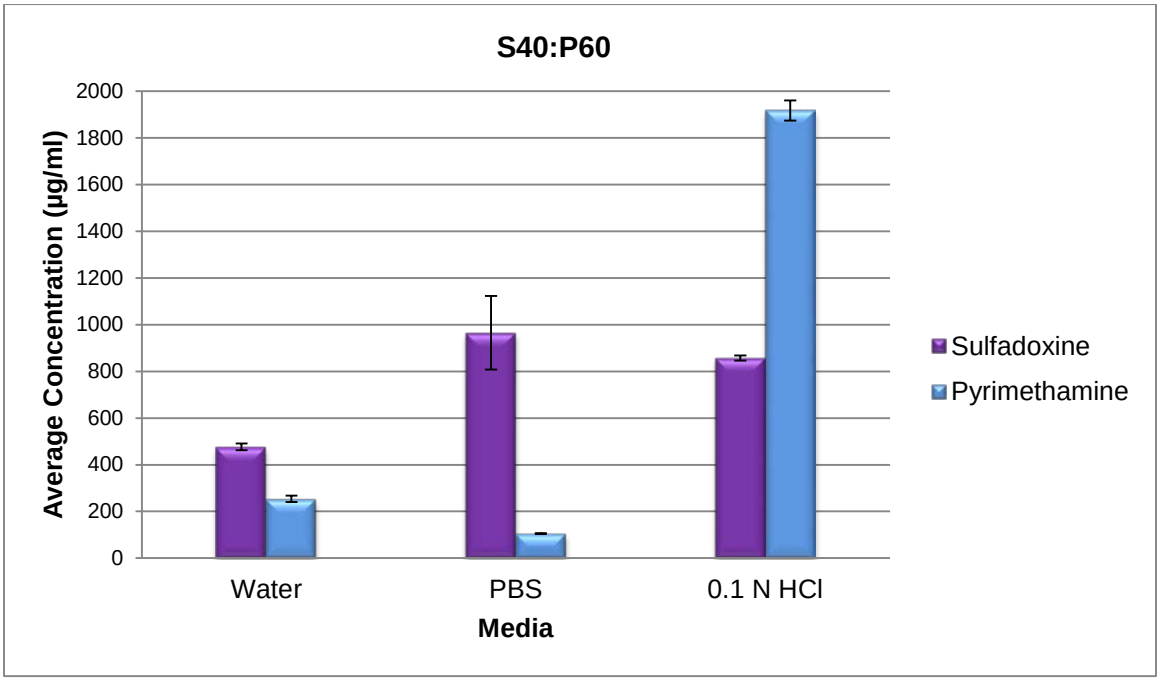


**Figure 4.12:** Graph showing the solubility concentrations (*µg/ml*) of sulfadoxine and pyrimethamine determined from a %w/w ratio of S40:P60 combination in distilled water, PBS and 0.1 N HCl.

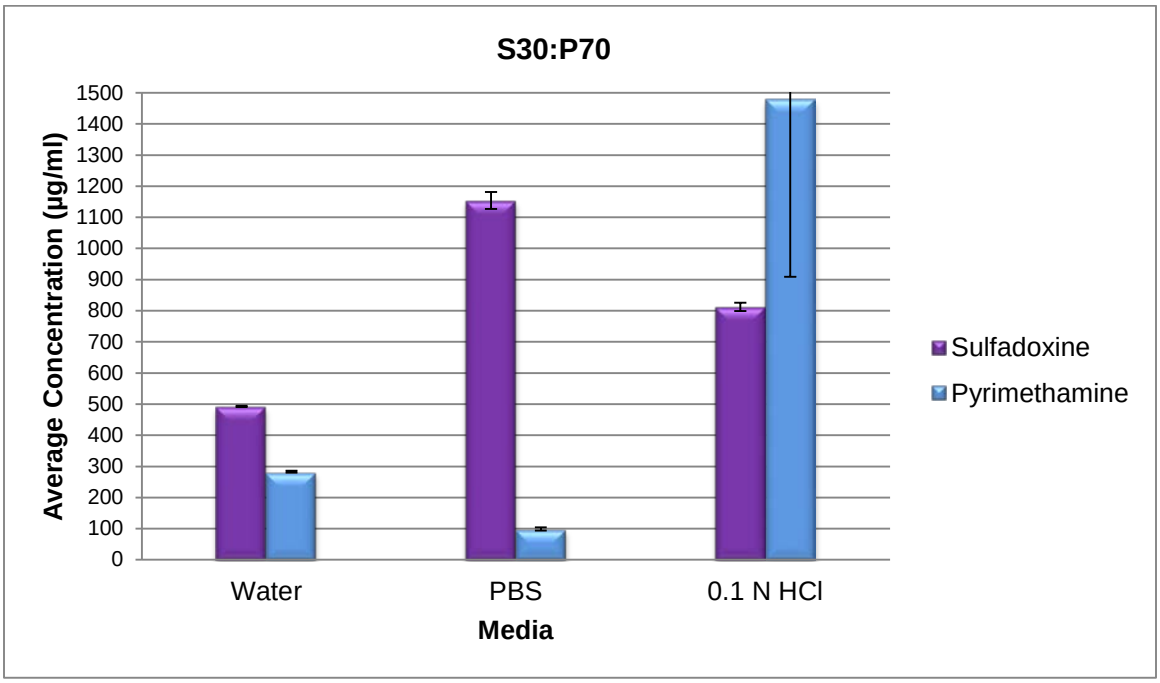


**Figure 4.13:** Graph depicting the solubility concentrations (*µg/ml*) of sulfadoxine and pyrimethamine determined from a %w/w ratio of S30:P70 combination in distilled water, PBS and 0.1 N HCl.

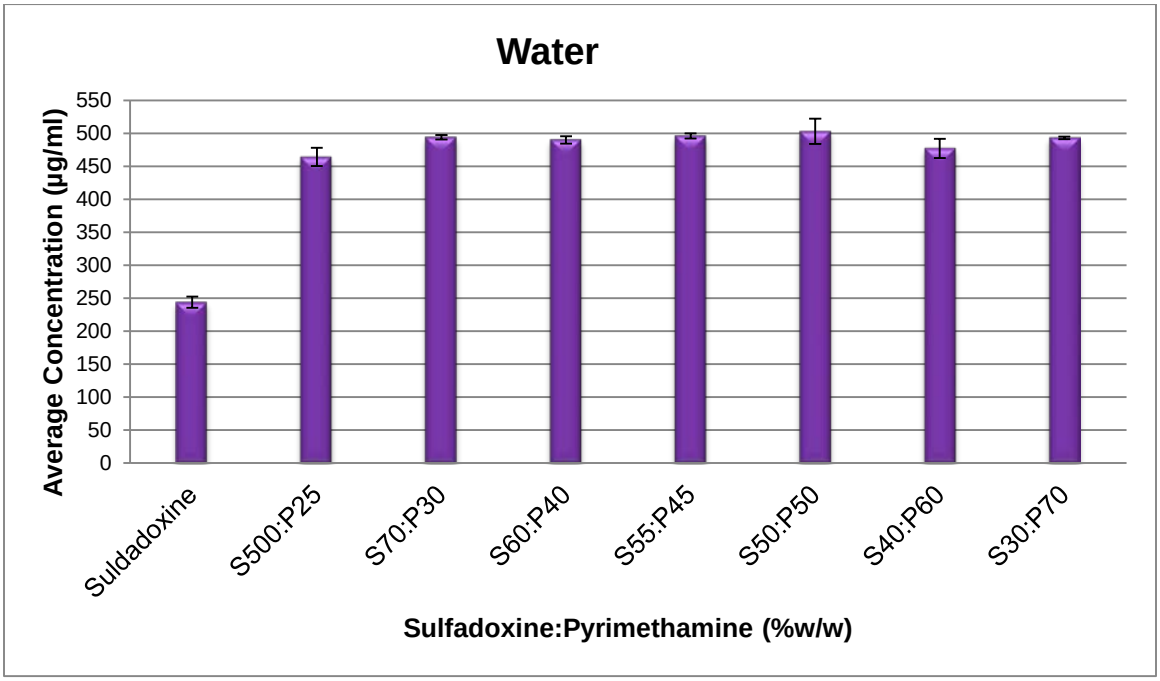


**Figure 4.14:** A comparative graph showing the determined average concentration (*µg/ml*) results of sulfadoxine in distilled water, as determined from all the different %w/w combinations.

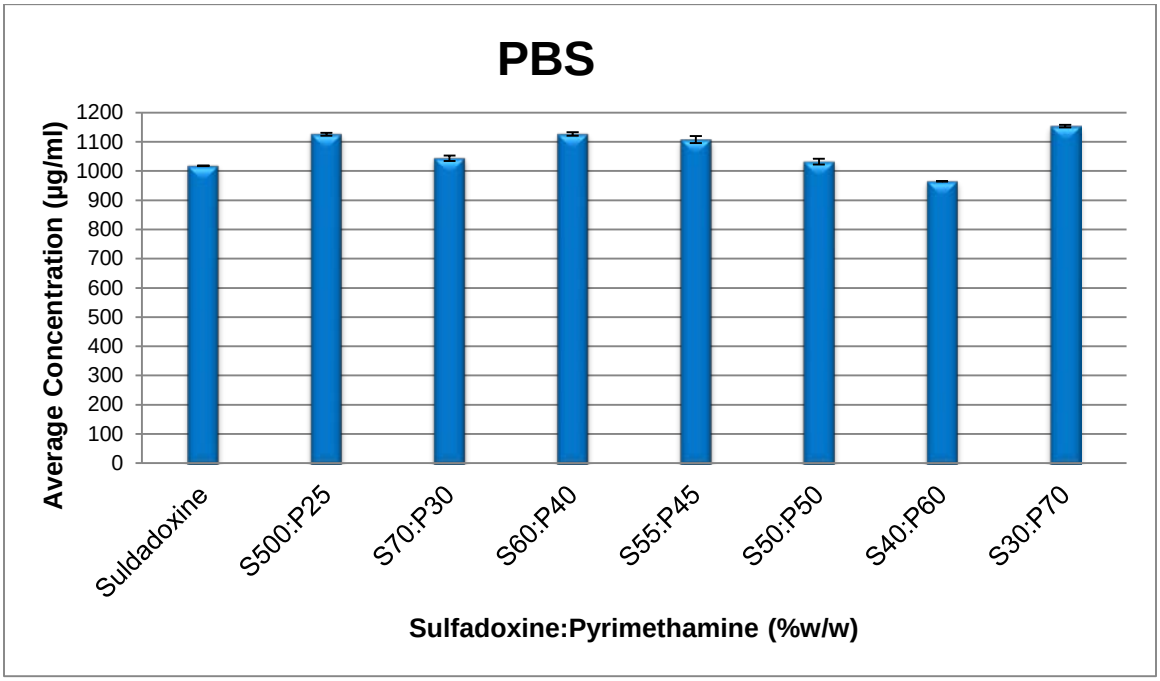


**Figure 4.15:** A comparative graph showing the determined average concentration (*µg/ml*) results of sulfadoxine in PBS, as determined from all the different %w/w combinations.

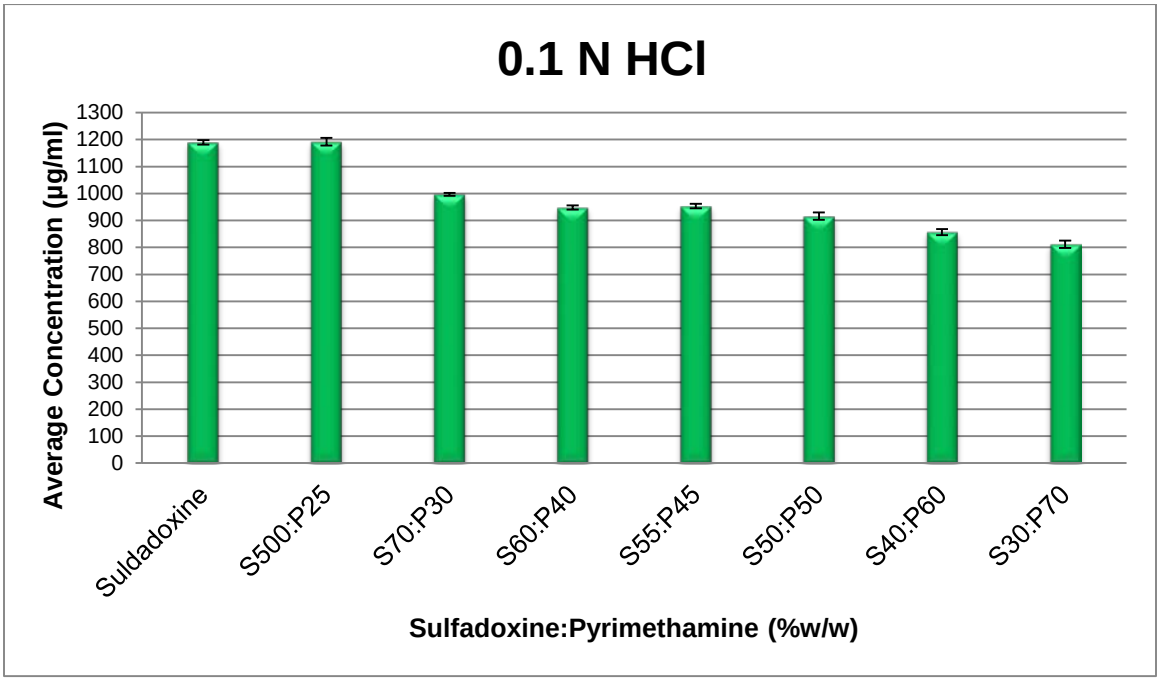


**Figure 4.16:** A graph showing a comparison of the determined sulfadoxine concentrations (*µg/ml*) in 0.1 N HCl, as determined from all the different %w/w combinations.

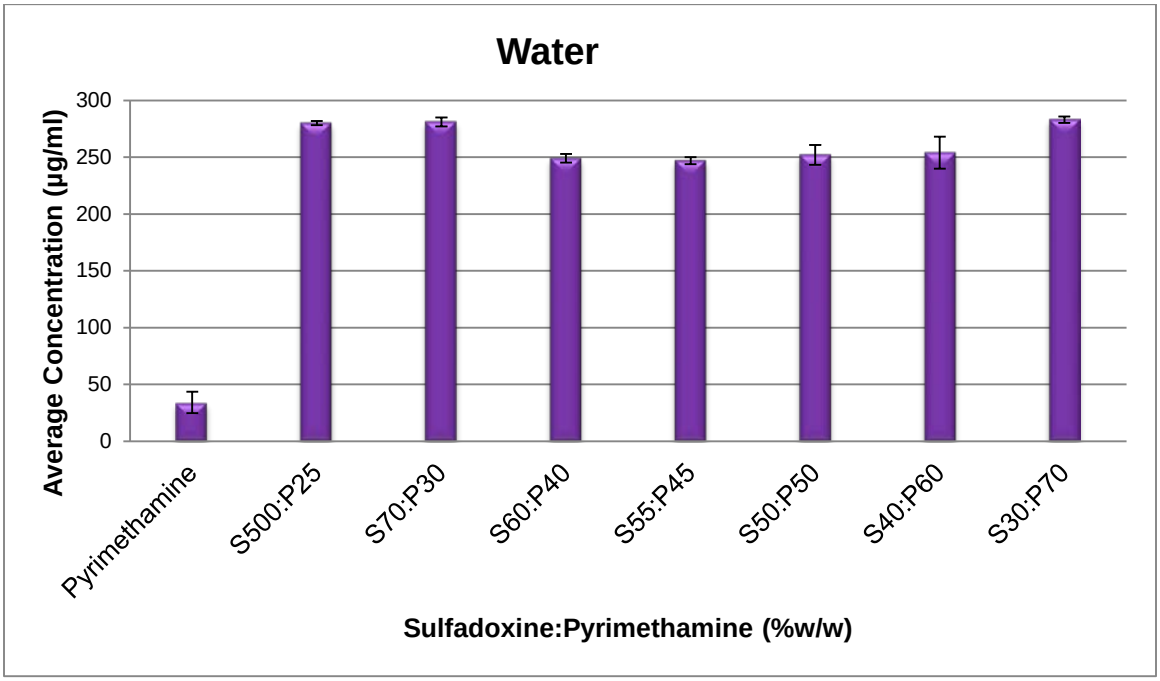


**Figure 4.17:** A comparative graph showing the determined average concentration (*µg/ml*) results of pyrimethamine in distilled water, as determined from all the different %w/w combinations.

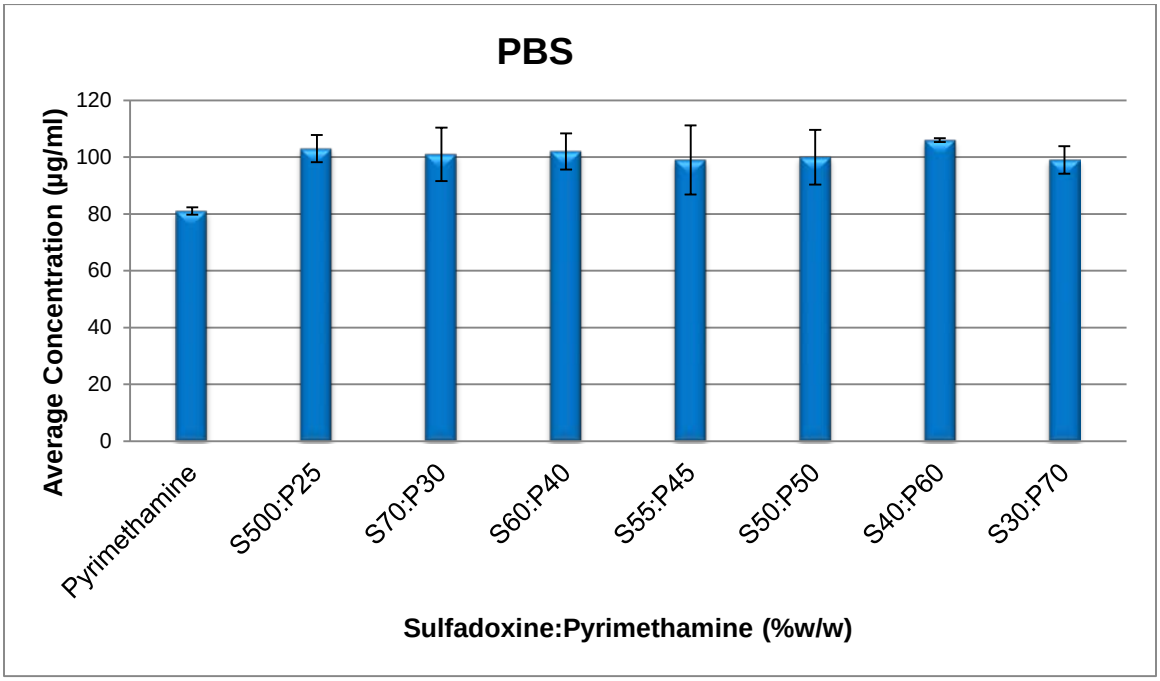


**Figure 4.18:** A comparative graph showing the determined average concentration (*µg/ml*) results of pyrimethamine in PBS, as determined from all the different %w/w combinations.

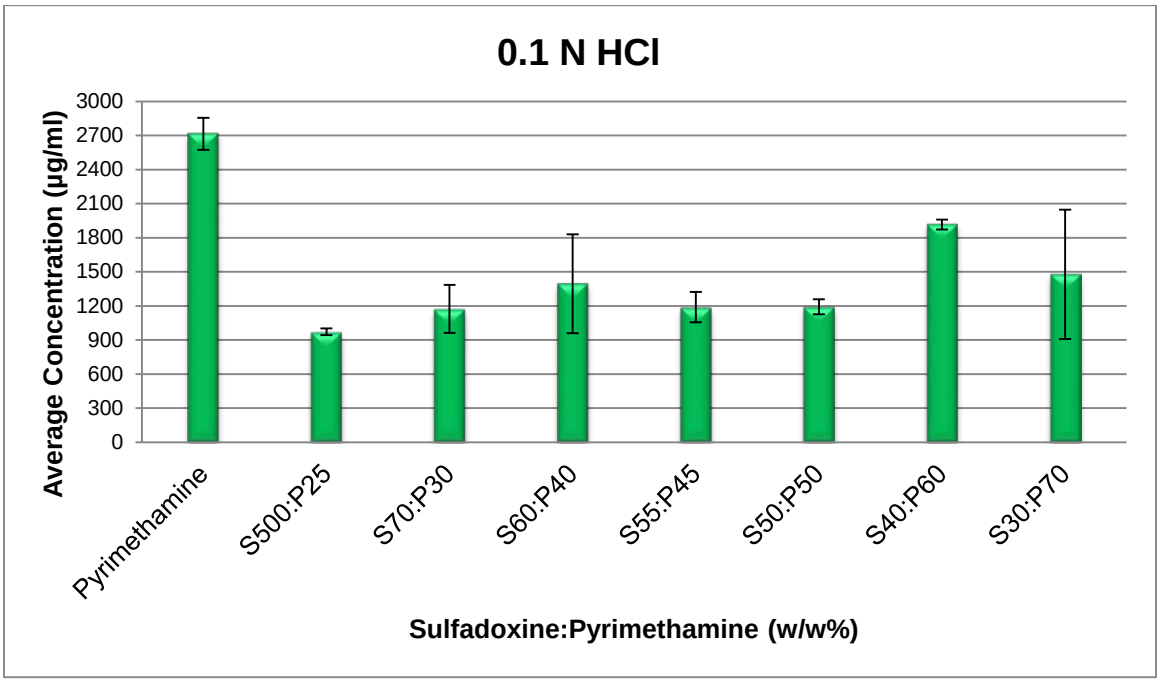


**Figure 4.19:** A graph showing a comparison of the determined pyrimethamine concentrations (*µg/ml*) in 0.1 N HCl, as determined from all the different %w/w combinations.

### 4.3.1 Discussion

Figures 4.5 and 4.6 depict the two single components in three different media. Sulfadoxine and pyrimethamine has the best solubility results in 0.1 N HCl with average concentrations of 1190 µg/ml and 2714 µg/ml respectively. Both of these components delivered the lowest solubility results in water with concentrations of 244 µg/ml and 34 µg/ml for sulfadoxine and pyrimethamine respectively.

Figure 4.7 indicates results obtained for the S500:P25 combination. In this combination both sulfadoxine and pyrimethamine performed the best in 0.1 N HCl with concentrations of 1192 µg/ml and 972 µg/ml respectively. This is possibly due to the fact that less pyrimethamine is available in the ratio.

For all the SP combinations (Figures 4.8 – 4.13) sulfadoxine had the highest solubility results in PBS and the lowest in water. The sulfadoxine concentration in PBS ranges from 965 to 1154 µg/ml and 464 – 503 µg/ml in water with no significant difference in the solubility for the different ratios in both PBS and water.

For all the SP combinations (Figures 4.8 – 4.13) pyrimethamine had the highest solubility results in 0.1 N HCl and ranges from 972 – 1917 µg/ml. The lowest results obtained for pyrimethamine was in PBS with a range from 81 - 106 µg/ml. There was no significant increase of the solubility of pyrimethamine in PBS.

Figures 4.14 and 4.15 depict the comparative results of the sulfadoxine single component and different SP combinations in water and PBS. These results indicate that when sulfadoxine is in combination with pyrimethamine the solubility of sulfadoxine in water increases significantly while in PBS no significant changes are obvious. No fixed pattern is present in these two figures when the results of the different concentrations are compared with the different SP combinations.

Figures 4.17 and 4.18 depict the comparative results of the pyrimethamine single component and different SP combinations in water and PBS. The solubility of pyrimethamine when in combination with sulfadoxine is significantly higher in water than that of the single component. The solubility results or PBS indicate a slight increase for the pyrimethamine in combination compared to the single component.

Figures 4.16 and 4.19 clearly indicate that when these two actives are in combination; the solubility of sulfadoxine and pyrimethamine in 0.1 N HCl decreases significantly.

The high percentage RSDs obtained during some of the studies could be attributed to low concentration values and experimental error.

### 4.3.2 Conclusion

The overall results emphasised that when sulfadoxine and pyrimethamine are in combination, the solubility of both of these drugs increase in water and PBS but the solubility of both of these two actives decreases in 0.1 N HCl when compared to the solubility concentrations of the single components.

## 4.4 Dissolution

Dissolution testing was performed on the different combinations (Table 3.1) in distilled water, PBS (pH 6.8) and 0.1 N HCl. Results are presented in Tables 4.15 - 4.41. The USP Q-time requirement for SP fixed dose tablet combination of 30 minutes is highlighted in each table (USP, 2016).

Results of the dissolution studies done in distilled water are presented in Tables 4.15 – 4.23.

**Table 4.15:** Dissolution percentage of sulfadoxine single component in distilled water

| Time (min) | % Dissolution | % RSD |
|---|---|---|
| **10** | 28.1 | **16.52** |
| **20** | 50.8 | **5.82** |
| **30** | 60.5 | **3.89** |
| **45** | 67.1 | **3.46** |
| **60** | 71.1 | **3.30** |

**Table 4.16:** Dissolution percentage of pyrimethamine single component in distilled water

| Time (min) | % Dissolution | % RSD |
|---|---|---|
| **10** | 0.72 | **10.34** |
| **20** | 2.9 | **10.78** |
| **30** | 5.0 | **13.07** |
| **45** | 7.5 | **11.81** |
| **60** | 9.6 | **10.03** |

**Table 4.17:** Dissolution percentage of S500:P25 combination in distilled water

| | **Sulfadoxine** | | **Pyrimethamine** | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 29.6 | **11.41** | 28.7 | **9.46** |
| **20** | 52.8 | **3.10** | 63.6 | **11.51** |
| **30** | 63.0 | **3.16** | 74.6 | **7.37** |
| **45** | 69.4 | **2.55** | 90.7 | **8.95** |
| **60** | 73.1 | **1.94** | 95.1 | **4.65** |

**Table 4.18:** Dissolution percentage of S30:P70 combination in distilled water

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 7.9 | **23.9** | 2.1 | **20.76** |
| **20** | 22.8 | **7.30** | 6.8 | **7.54** |
| **30** | 38.0 | **43.25** | 15.8 | **54.21** |
| **45** | 47.3 | **15.13** | 19.3 | **20.31** |
| **60** | 53.4 | **16.09** | 24.0 | **25.29** |

**Table 4.19:** Dissolution percentage of S40:P60 combination in distilled water

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 7.4 | **31.43** | 1.5 | **31.42** |
| **20** | 26.8 | **14.83** | 7.5 | **14.83** |
| **30** | 38.6 | **7.33** | 12.1 | **7.33** |
| **45** | 46.4 | **5.78** | 16.5 | **5.78** |
| **60** | 51.2 | **6.52** | 20.7 | **6.52** |

**Table 4.20:** Dissolution percentage of S50:P50 combination in distilled water

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 14.0 | **17.79** | 3.8 | **43.09** |
| **20** | 39.5 | **10.53** | 13.4 | **11.21** |
| **30** | 54.8 | **15.43** | 20.7 | **16.15** |
| **45** | 62.9 | **12.59** | 27.1 | **12.11** |
| **60** | 66.3 | **12.89** | 34.2 | **14.35** |

**Table 4.21:** Dissolution percentage of S55:P45 combination in distilled water

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 21.0 | **17.76** | 5.4 | **22.09** |
| **20** | 51.8 | **10.96** | 17.2 | **12.52** |
| **30** | 63.2 | **10.77** | 24.6 | **11.49** |
| **45** | 73.6 | **9.55** | 33.1 | **9.00** |
| **60** | 79.3 | **9.58** | 39.6 | **9.16** |

**Table 4.22:** Dissolution percentage of S60:P40 combination in distilled water

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 10.6 | **29.15** | 4.5 | **27.53** |
| **20** | 33.2 | **16.13** | 16.2 | **18.33** |
| **30** | 48.0 | **6.98** | 26.7 | **8.71** |
| **45** | 58.4 | **4.21** | 37.5 | **5.32** |
| **60** | 65.1 | **3.23** | 45.0 | **7.65** |

**Table 4.23:** Dissolution percentage of S70:P30 (sulfadoxine : pyrimethamine) combination in distilled water

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 15.4 | **15.54** | 8.6 | **16.86** |
| **20** | 44.4 | **6.77** | 25.2 | **9.43** |
| **30** | 58.3 | **4.68** | 37.4 | **5.79** |
| **45** | 66.9 | **4.74** | 49.1 | **5.40** |
| **60** | 72.5 | **3.87** | 56.8 | **6.77** |

Results of the dissolution studies done in PBS (pH 6.8) are presented in Tables 4.24 – 4.32.

**Table 4.24:** Dissolution percentage of sulfadoxine single component in PBS

| Time (min) | % Dissolution | % RSD |
|---|---|---|
| **10** | 45.3 | **21.41** |
| **20** | 71.1 | **35.35** |
| **30** | 87.0 | **3.23** |
| **45** | 89.0 | **3.26** |
| **60** | 90.1 | **3.36** |

**Table 4.25:** Dissolution percentage of pyrimethamine single component in PBS

| Time (min) | % Dissolution | % RSD |
|---|---|---|
| **10** | 0.90 | **35.56** |
| **20** | 3.2 | **47.85** |
| **30** | 7.9 | **25.15** |
| **45** | 11.5 | **24.71** |
| **60** | 14.1 | **23.05** |

**Table 4.26:** Dissolution percentage of S500:P25 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 56.7 | **27.36** | 26.0 | **26.78** |
| **20** | 79.5 | **36.58** | 21.3 | **49.07** |
| **30** | 92.0 | **3.80** | 33.4 | **7.40** |
| **45** | 93.5 | **2.40** | 40.4 | **7.68** |
| **60** | 94.6 | **1.55** | 44.8 | **7.80** |

**Table 4.27:** Dissolution percentage of S30:P70 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 9.3 | **33.84** | 1.5 | **26.26** |
| **20** | 23.2 | **37.20** | 4.7 | **35.69** |
| **30** | 40.0 | **13.23** | 13.0 | **41.33** |
| **45** | 46.7 | **9.18** | 15.7 | **9.23** |
| **60** | 51.7 | **9.09** | 19.2 | **9.97** |

**Table 4.28:** Dissolution percentage of S40:P60 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 8.6 | **58.25** | 1.9 | **36.20** |
| **20** | 24.6 | **38.55** | 6.0 | **40.90** |
| **30** | 39.0 | **18.32** | 12.7 | **25.75** |
| **45** | 44.6 | **14.25** | 18.2 | **18.25** |
| **60** | 48.3 | **16.54** | 22.3 | **16.68** |

**Table 4.29:** Dissolution percentage of S50:P50 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 27.3 | **15.90** | 4.8 | **20.31** |
| **20** | 55.2 | **8.37** | 13.6 | **11.17** |
| **30** | 66.4 | **88.65** | 20.3 | **8.31** |
| **45** | 75.6 | **12.60** | 27.6 | **8.55** |
| **60** | 88.0 | **8.89** | 33.5 | **7.13** |

**Table 4.30:** Dissolution percentage of S55:P45 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 22.8 | **10.98** | 3.9 | **13.22** |
| **20** | 51.8 | **7.61** | 12.9 | **8.33** |
| **30** | 65.0 | **7.43** | 21.0 | **10.13** |
| **45** | 77.2 | **9.56** | 28.3 | **9.97** |
| **60** | 88.4 | **18.39** | 35.4 | **12.56** |

**Table 4.31:** Dissolution percentage of S60:P40 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 14.6 | **60.26** | 2.9 | **53.53** |
| **20** | 37.9 | **39.17** | 11.4 | **49.07** |
| **30** | 54.7 | **7.43** | 18.8 | **6.74** |
| **45** | 64.2 | **9.09** | 25.2 | **6.34** |
| **60** | 69.5 | **10.42** | 35.5 | **7.80** |

**Table 4.32:** Dissolution percentage of S70:P30 combination in PBS

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 19.9 | 50.38 | 4.6 | 46.07 |
| **20** | 37.3 | **34.87** | 17.5 | **67.89** |
| **30** | 58.0 | **11.62** | 21.5 | **16.15** |
| **45** | 67.5 | **9.53** | 31.1 | **14.76** |
| **60** | 73.3 | **6.41** | 37.6 | **10.34** |

Results of the dissolution studies done in 0.1 N HCl are presented in Tables 4.33 – 4.41.

**Table 4.33:** Dissolution percentage of sulfadoxine single component in 0.1 N HCl

| Time (min) | % Dissolution | % RSD |
|---|---|---|
| **10** | 58.8 | **18.08** |
| **20** | 69.0 | **46.55** |
| **30** | 86.1 | **7.32** |
| **45** | 88.6 | **6.92** |
| **60** | 91.5 | **5.84** |

**Table 4.34:** Dissolution percentage of pyrimethamine single component 0.1 N HCl

| Time (min) | % Dissolution | % RSD |
|---|---|---|
| **10** | 20.1 | **65.72** |
| **20** | 37.0 | **43.43** |
| **30** | 47.4 | **33.89** |
| **45** | 57.2 | **28.84** |
| **60** | 65.3 | **25.64** |

**Table 4.35:** Dissolution percentage of S500:P25 combination 0.1 N HCl

| | **Sulfadoxine** | | **Pyrimethamine** | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 26.9 | **85.21** | 28.1 | **73.49** |
| **20** | 49.7 | **64.17** | 41.0 | **27.70** |
| **30** | 58.0 | **49.52** | 63.2 | **50.92** |
| **45** | 83.7 | **24.02** | 67.8 | **26.04** |
| **60** | 91.6 | **19.94** | 91.9 | **12.71** |

**Table 4.36:** Dissolution percentage of S30:P70 combination 0.1 N HCl

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 17.9 | **24.27** | 16.4 | **19.68** |
| **20** | 32.2 | **23.90** | 24.9 | **53.02** |
| **30** | 51.5 | **28.37** | 41.6 | **19.94** |
| **45** | 57.5 | **16.87** | 52.7 | **16.28** |
| **60** | 70.2 | **16.55** | 64.5 | **16.12** |

**Table 4.37:** Dissolution percentage of S40:P60 combination 0.1 N HCl

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 31.4 | **39.29** | 32.0 | **49.83** |
| **20** | 44.9 | **48.46** | 45.7 | **22.16** |
| **30** | 62.5 | **19.30** | 60.4 | **17.14** |
| **45** | 76.9 | **13.54** | 65.6 | **43.36** |
| **60** | 88.9 | **9.60** | 85.7 | **9.42** |

**Table 4.38:** Dissolution percentage of S50:P50 combination 0.1 N HCl

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 30.6 | **26.53** | 27.6 | **42.23** |
| **20** | 42.5 | **49.35** | 46.3 | **50.68** |
| **30** | 60.4 | **15.23** | 61.0 | **14.57** |
| **45** | 71.3 | **13.45** | 72.5 | **13.12** |
| **60** | 78.1 | **13.76** | 79.5 | **13.28** |

**Table 4.39:** Dissolution percentage of S55:P45 combination 0.1 N HCl

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 32.3 | **71.01** | 28.1 | **73.49** |
| **20** | 49.7 | **64.16** | 45.7 | **44.18** |
| **30** | 58.0 | **57.53** | 59.1 | **64.54** |
| **45** | 83.7 | **24.01** | 67.8 | **26.03** |
| **60** | 91.6 | **19.94** | 74.6 | **21.30** |

**Table 4.40:** Dissolution percentage of S60:P40 combination 0.1 N HCl

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 33.5 | **23.24** | 35.1 | **21.29** |
| **20** | 49.0 | **35.50** | 48.7 | **34.69** |
| **30** | 70.0 | **12.36** | 69.2 | **10.56** |
| **45** | 83.4 | **5.31** | 82.0 | **5.48** |
| **60** | 90.5 | **5.30** | 88.6 | **5.24** |

**Table 4.41:** Dissolution percentage of S70:P30 combination 0.1 N HCl

| | Sulfadoxine | | Pyrimethamine | |
|---|---|---|---|---|
| **Time (min)** | **% Dissolution** | **%RSD** | **% Dissolution** | **%RSD** |
| **10** | 67.2 | **22.87** | 73.9 | **22.83** |
| **20** | 69.2 | **49.40** | 85.1 | **16.17** |
| **30** | 86.9 | **12.59** | 90.0 | **11.54** |
| **45** | 91.5 | **7.31** | 95.2 | **6.29** |
| **60** | 95.2 | **3.35** | 99.1 | **2.84** |

Dissolution results are presented as comparative profiles in Figures 4.20 – 4.25.

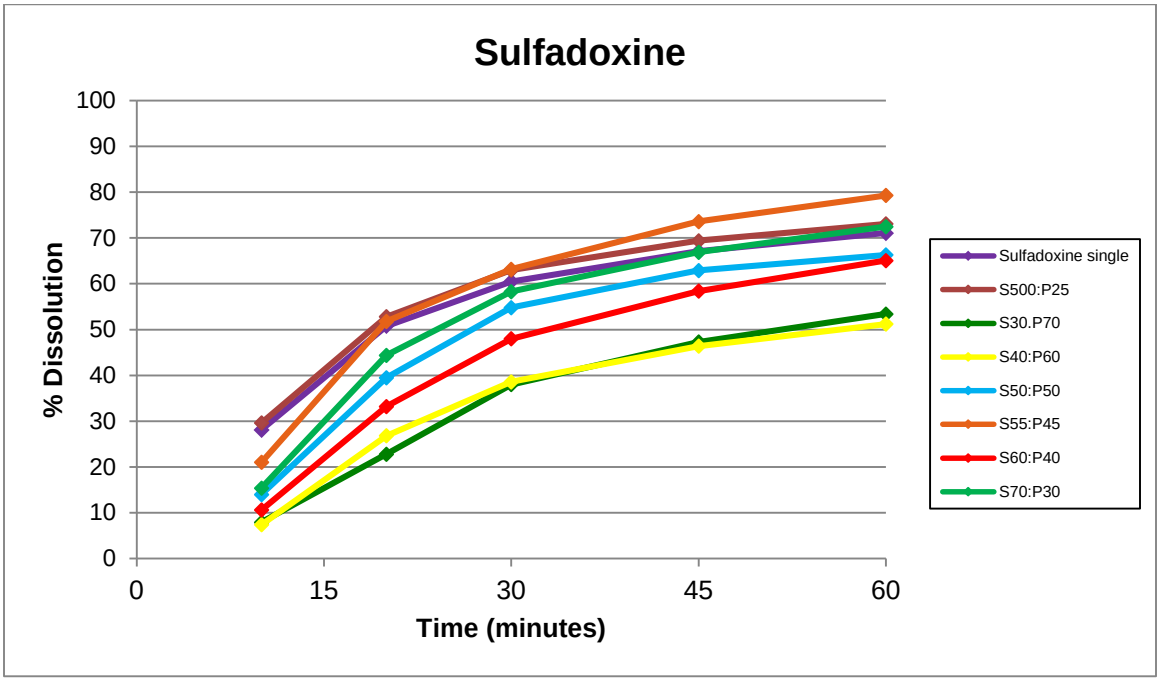


**Figure 4.20:** Comparative dissolution results for sulfadoxine in distilled water.

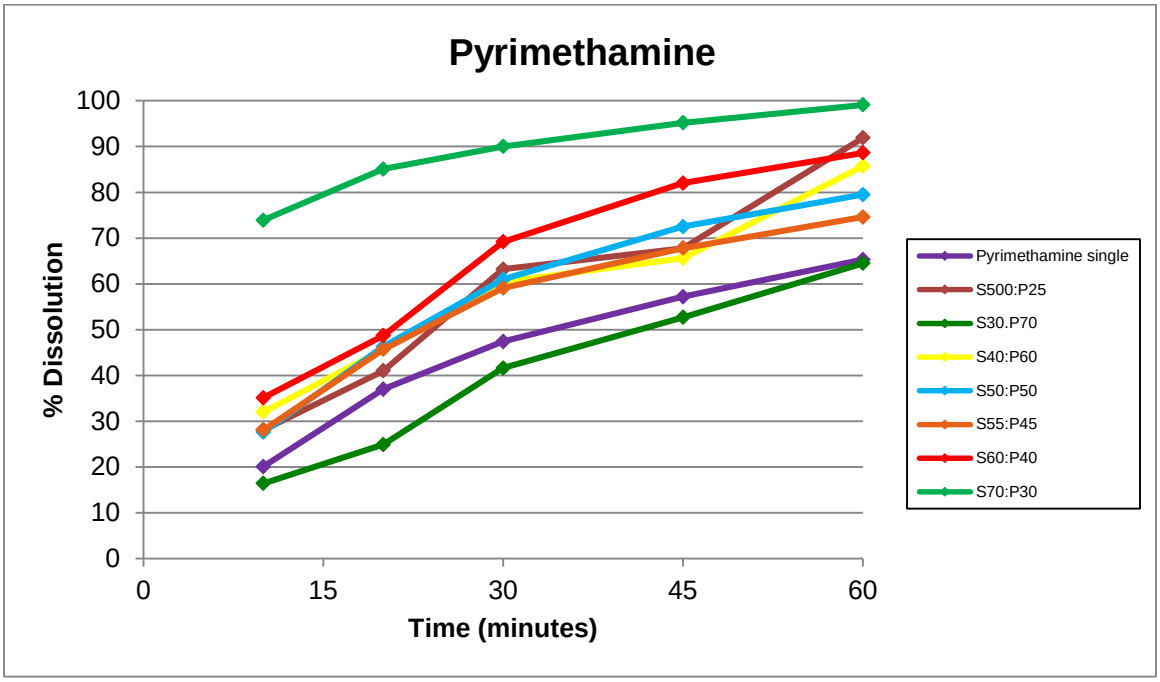


**Figure 4.21:** Comparative dissolution results for pyrimethamine in distilled water.

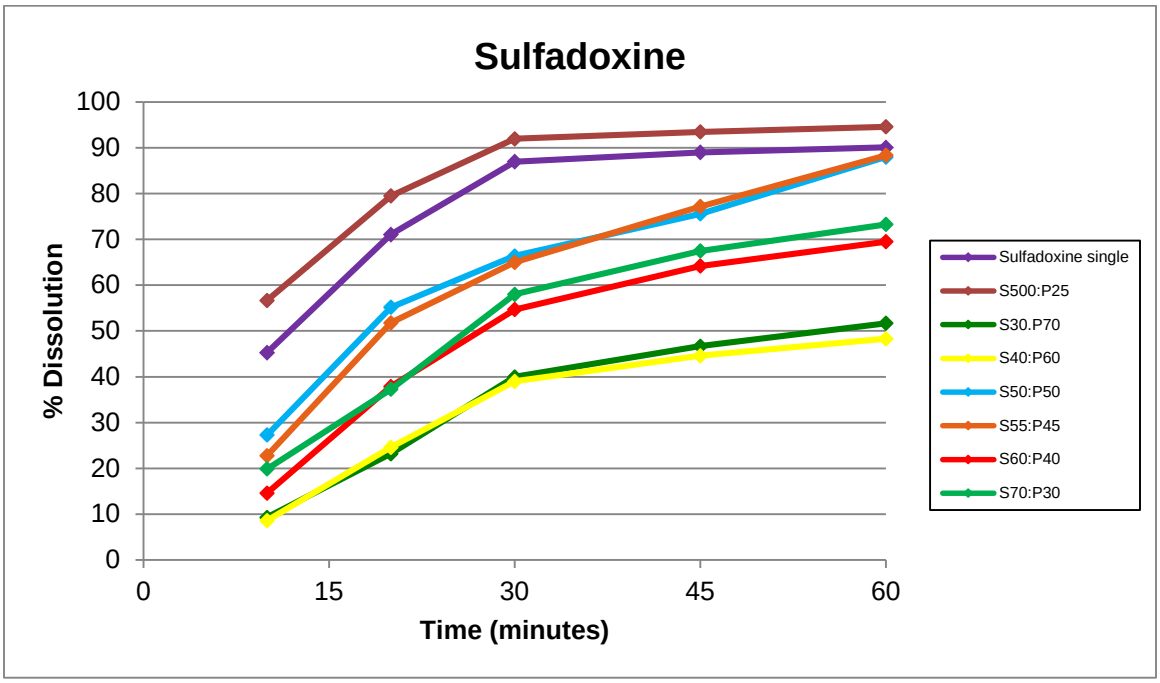


**Figure 4.22** Comparative dissolution results for sulfadoxine in PBS.

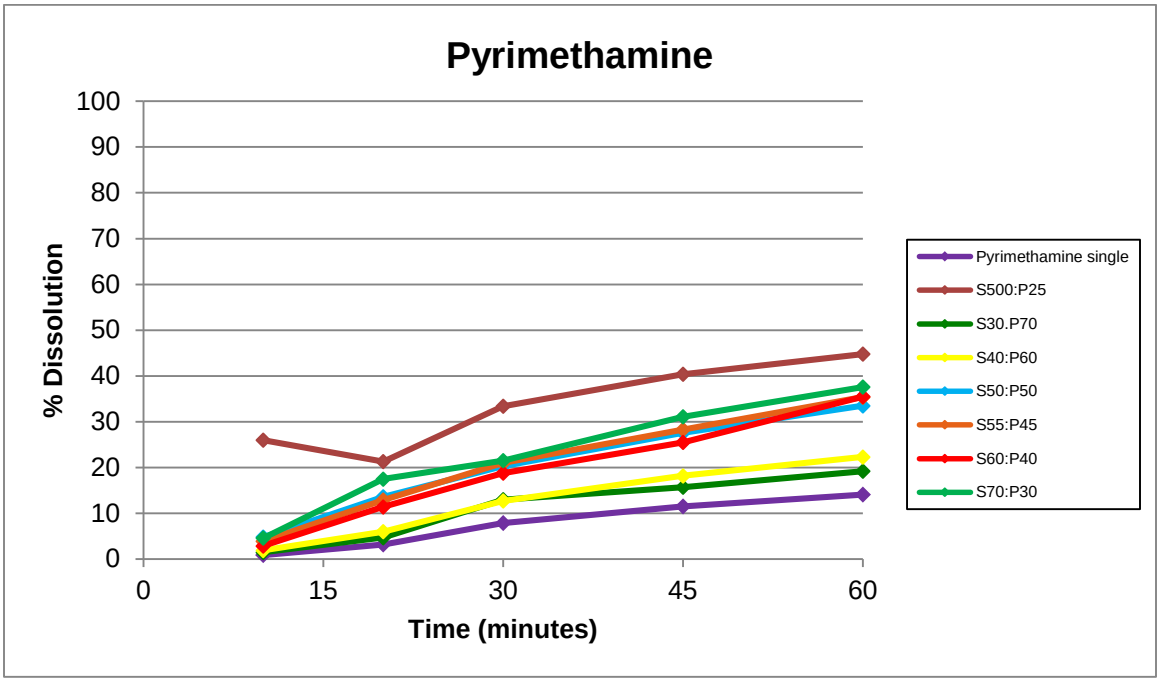


**Figure 4.23** Comparative dissolution results for pyrimethamine in PBS.

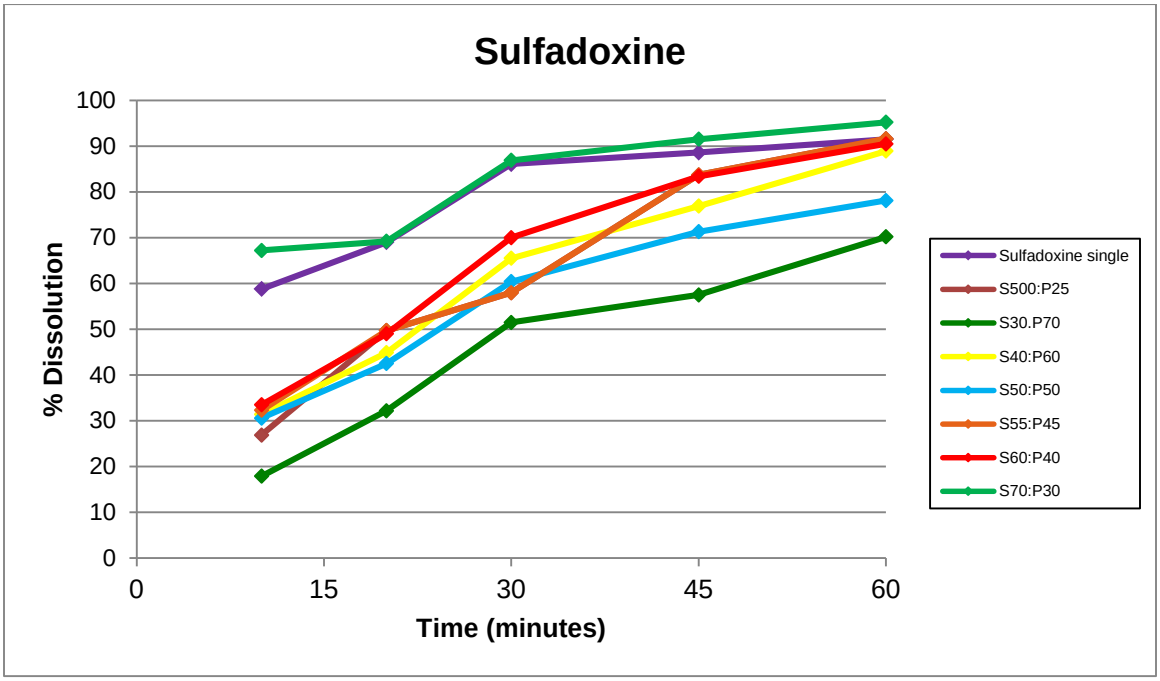


**Figure 4.24:** Comparative dissolution results for sulfadoxine in 0.1 N HCl.

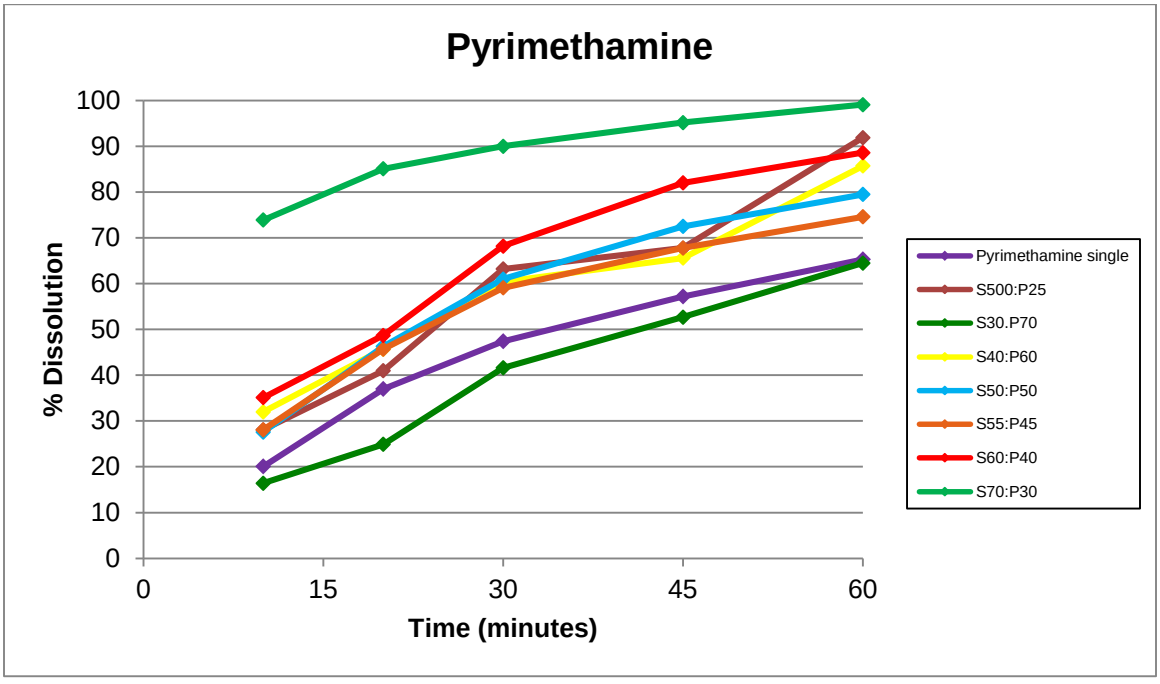


**Figure 4.25:** Comparative dissolution results for pyrimethamine in 0.1 N HCl.

The percentage dissolution of sulfadoxine and pyrimethamine reached in the three respective media are compared in Figures 4.26 – 4.27.

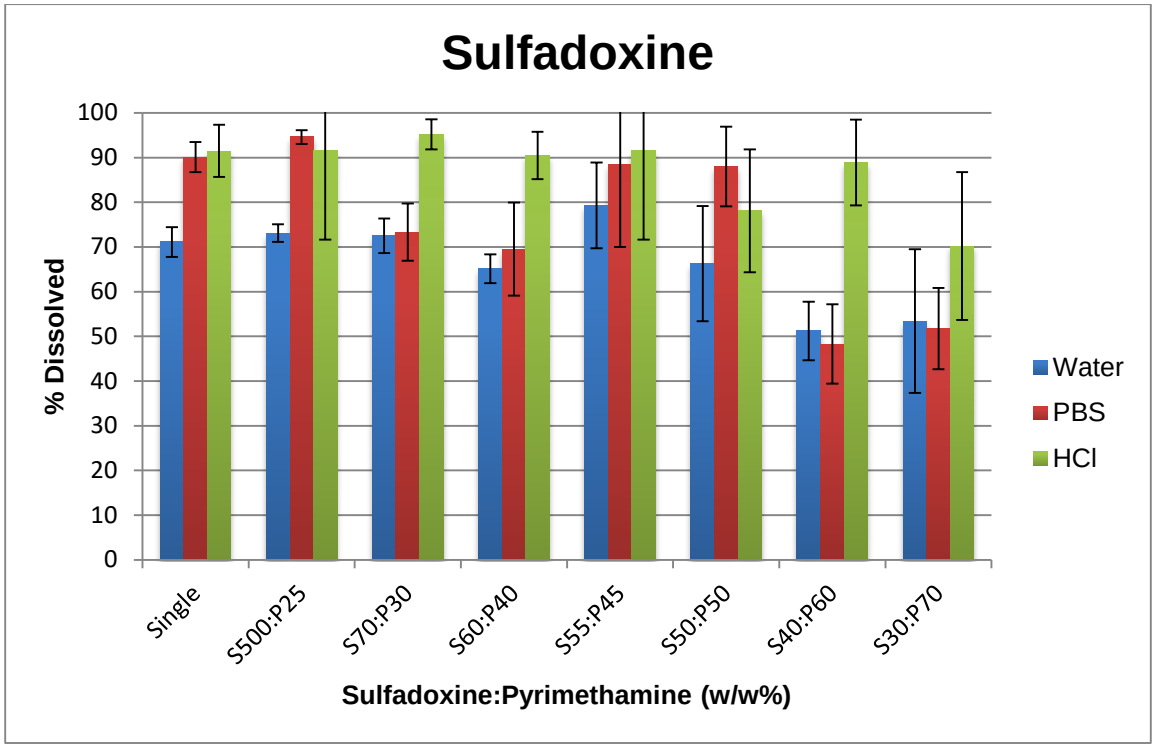


**Figure 4.26:** Combined graph of percentage dissolution after 30 minutes of sulfadoxine in distilled water, PBS and HCl.

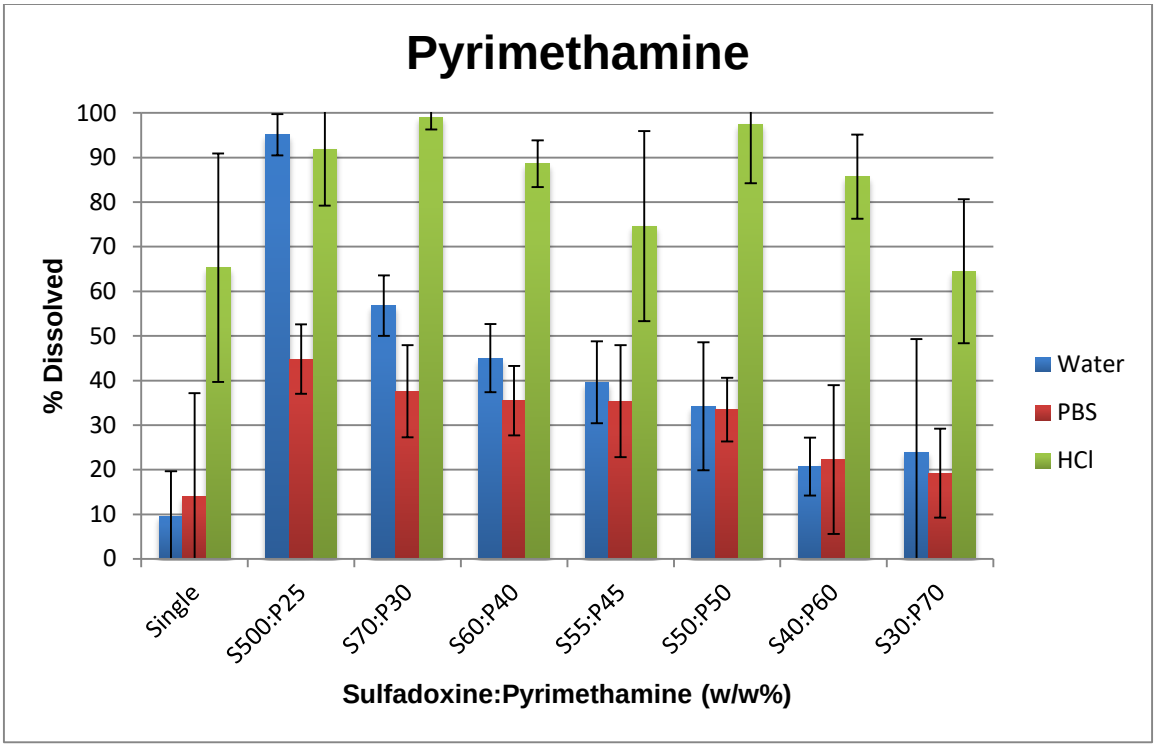


**Figure 4.27:** Combined graph of percentage dissolution of pyrimethamine after 30 minutes in distilled water, PBS and HCl.

### 4.4.1 Discussion

The results indicate that the USP requirements were met in only a few instances. It should however be noted that the USP requirement is for drug formulations and not for powder dissolutions.

For water, sulfadoxine reached 60% dissolution or higher in 30 minutes in three instances only: sulfadoxine as single component (60.5%), S500:P25 (63.0%) and S55:P45 (63.2%) combination. For pyrimethamine a percentage dissolution higher that 60% (74.6%) was reached for the tablet combination only.

For PBS, sulfadoxine reached 60% dissolution or higher in 30 minutes in four instances only: sulfadoxine as single component (87.0%), S500:P25 (92.0%), S50:P50 (66.4%) and S55:P45 (65%.0). For pyrimethamine a dissolution percentage higher than 60% was not obtained for any of the components that were tested.

For 0.1 N HCl, sulfadoxine reached 60% dissolution or higher in 30 minutes in five instances: sulfadoxine single components (86.1%), S40:P60 (62.5%), S50:P50 (60.4%), S60:P40 (70.0%) and S70:P30 (86.9%). For pyrimethamine a dissolution percentage higher than 60% was obtained in five instances: S500:P25 (63.2%), S40:P60 (60.4%), S50:P50 (61.0%), S60:P40 (68.2%) and S70:P30 (90.0%).

From Figures 4.20 – 4.27 it is clear that generally the percentage dissolution for both actives are better in 0.1 N HCl than for the other media. For sulfadoxine the difference in dissolution is not as pronounced as for the pyrimethamine.

The high percentage RSDs could be attributed to the low concentrations obtained during the analysis.

### 4.4.2 Conclusion

In contrast with solubility, the best results obtained for sulfadoxine and pyrimethamine were in 0.1 N HCl. The difference may be contributed to the fact that the powder mixes were filled in gelatin capsules and the volume of medium (1000 ml) used for dissolution, whilst solubility studies were performed on supersaturated solutions.

## 4.5 Differential Scanning Calorimetry (DSC)

Thermal analysis of a compound is regarded as a valuable technique to gain more information with regards to the physical properties thereof. It is also a tool typically used to determine if two or even

more components will interact or react with one another when in combination. The recorded thermograms with thermal events for each of the components (Table 3.1) or ratios are presented in Figures 4.28 – 4.35. In Figure 4.36 an overlay of all the ratios are presented.

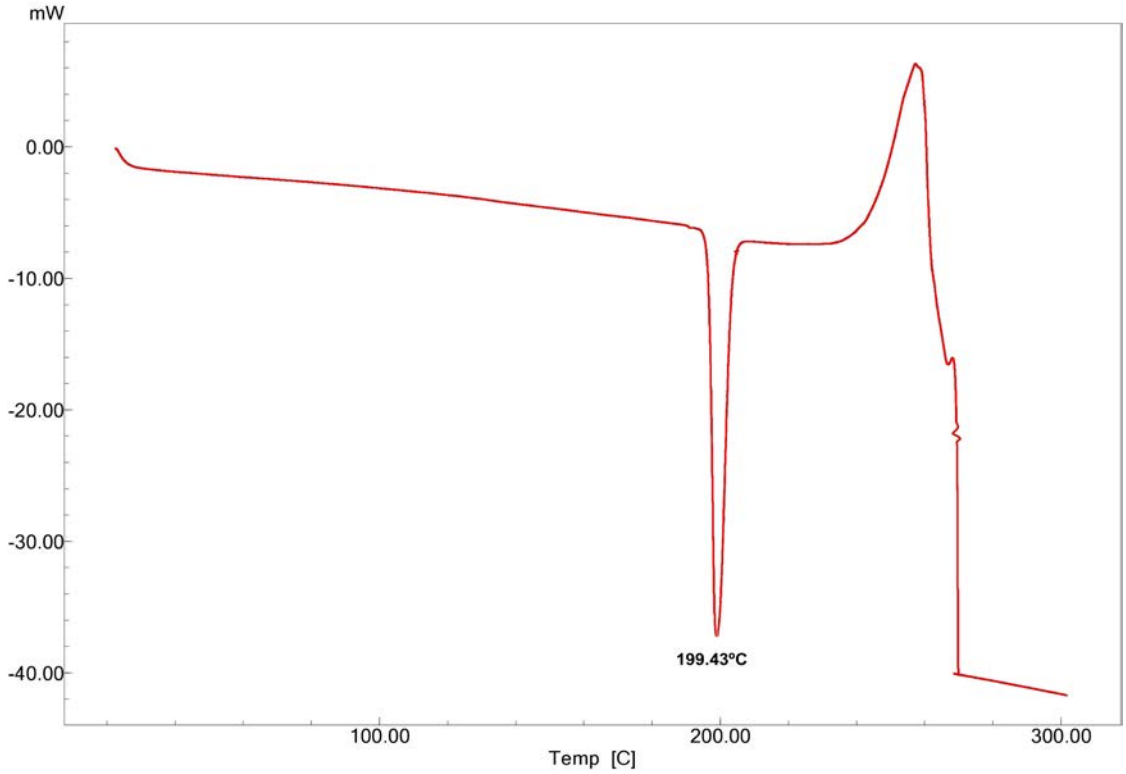


**Figure 4.28:** DSC thermogram obtained for sulfadoxine as single component.

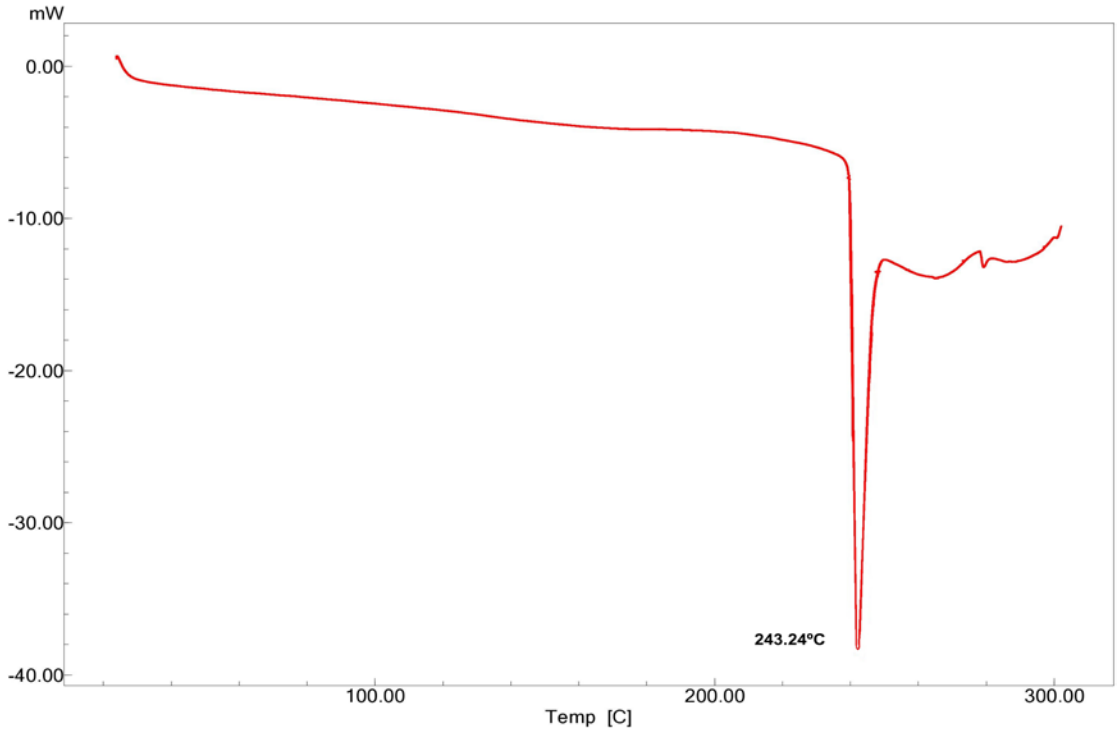


**Figure 4.29:** DSC thermogram obtained for pyrimethamine as single component.

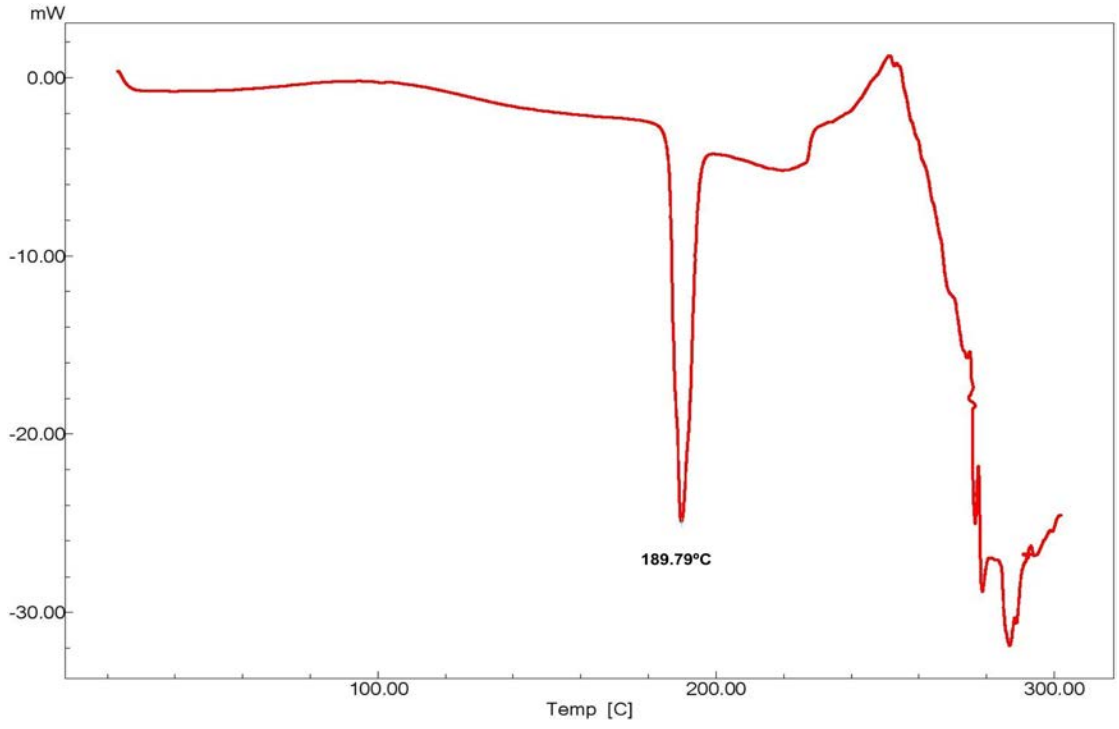


**Figure 4.30:** DSC thermogram obtained for sulfadoxine and pyrimethamine in a S70:P30 (% w/w) ratio.

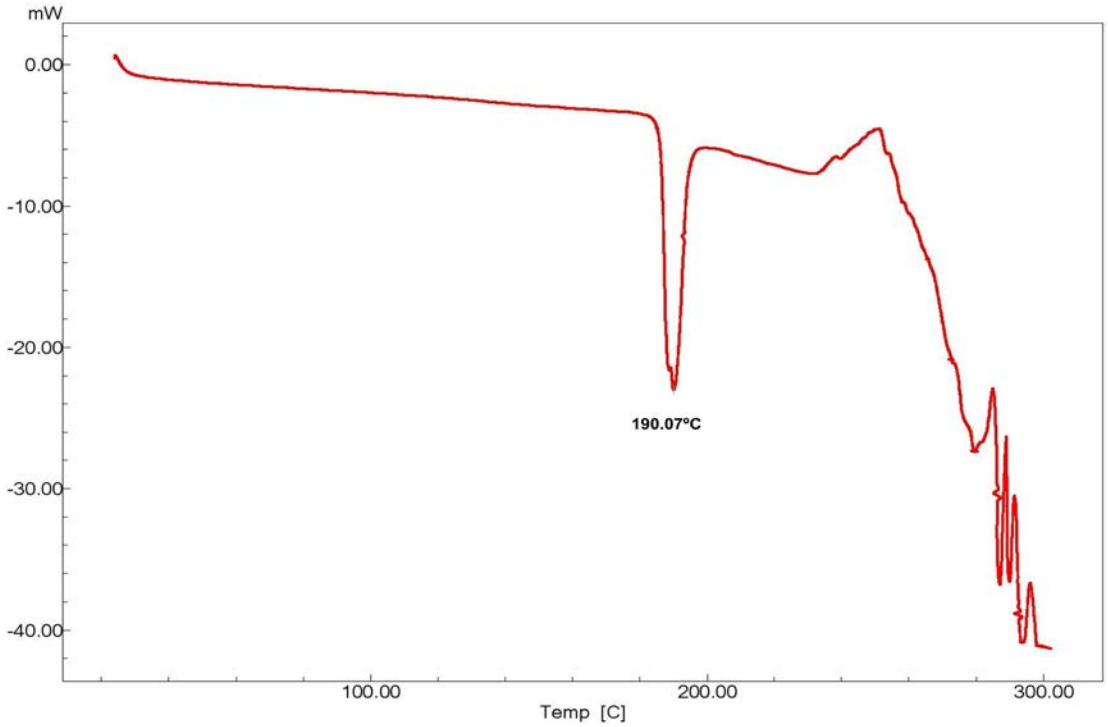


**Figure 4.31:** DSC thermogram obtained for sulfadoxine and pyrimethamine in a S60:P40 (% w/w) ratio.

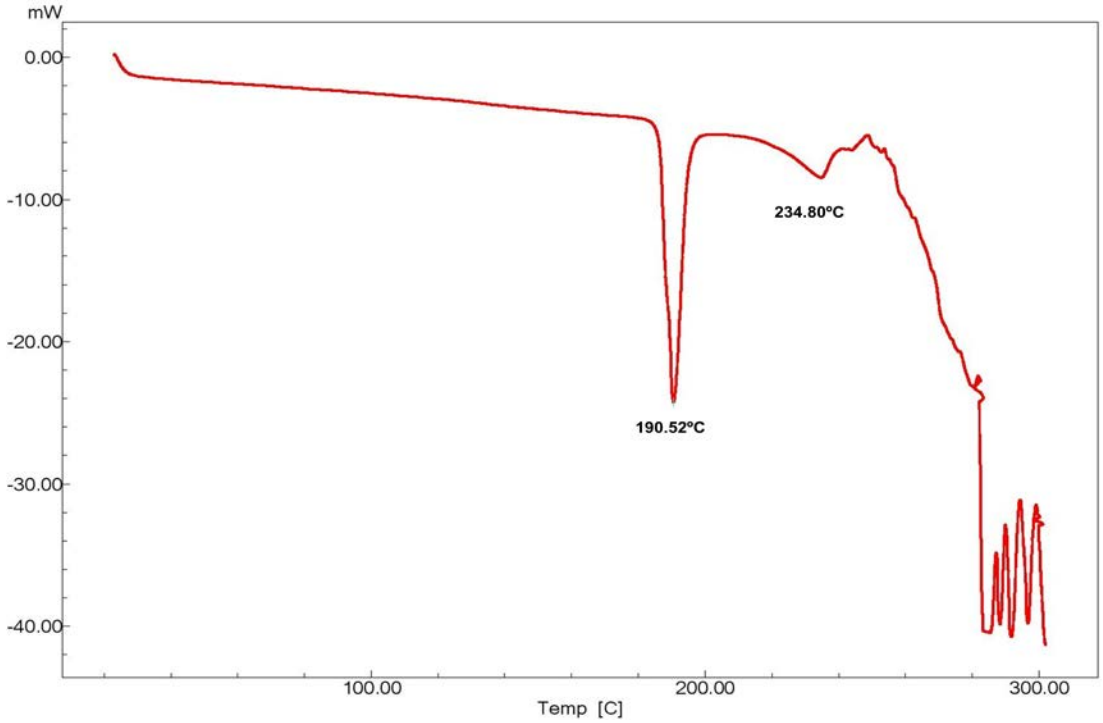


**Figure 4.32:** DSC thermogram obtained for sulfadoxine and pyrimethamine in a S55:P45 (% w/w) ratio.

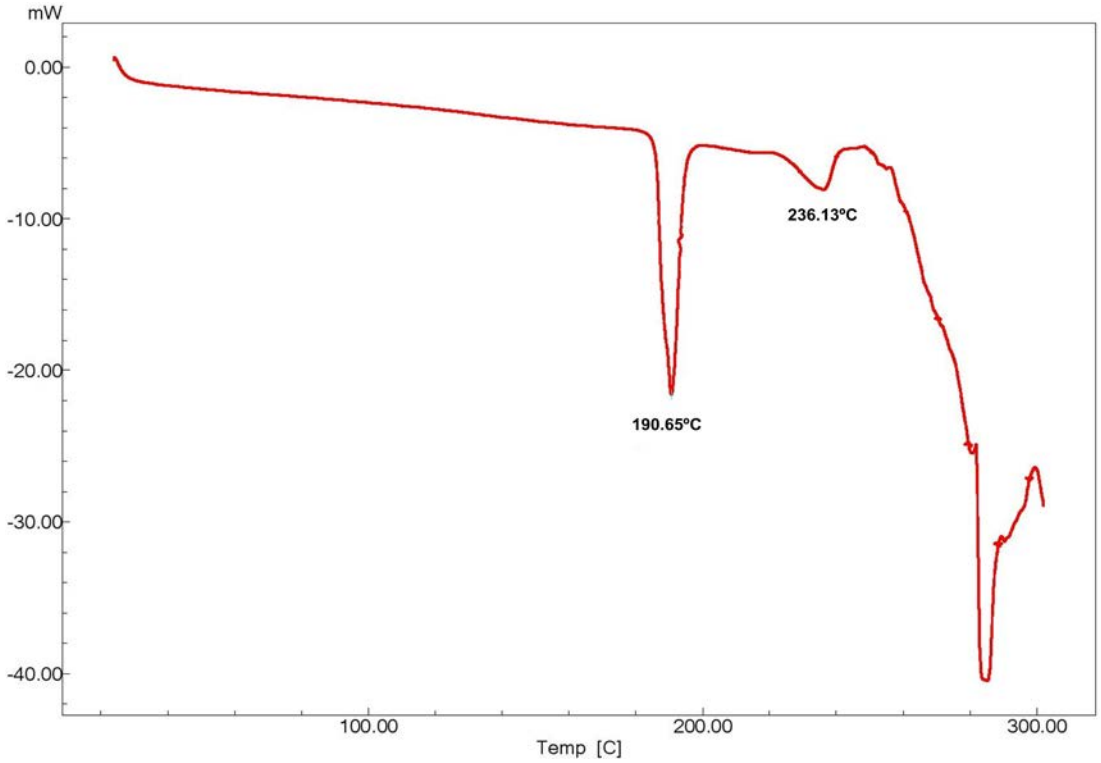


**Figure 4.33:** DSC thermogram obtained for sulfadoxine and pyrimethamine in a S50:P50 (% w/w) ratio.

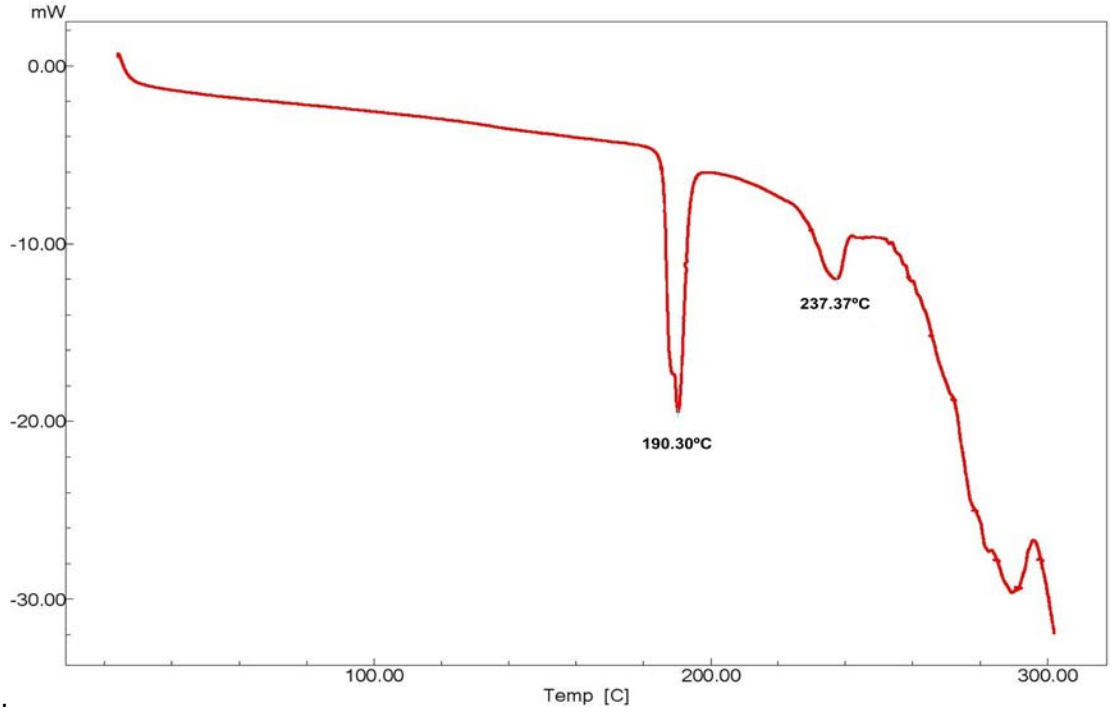


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**Figure 4.34:** DSC thermogram obtained for sulfadoxine and pyrimethamine in a S40:P60 (% w/w) ratio.

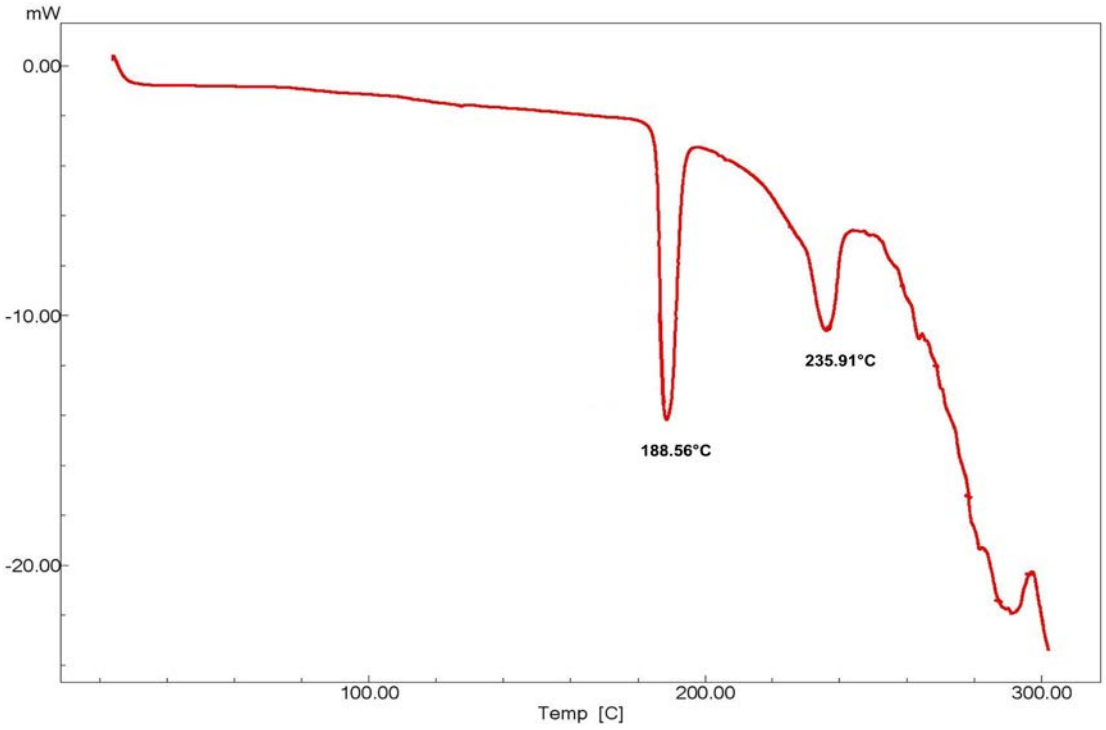


**Figure 4.35:** DSC thermogram obtained for sulfadoxine and pyrimethamine in a S30:P70 (% w/w) ratio.

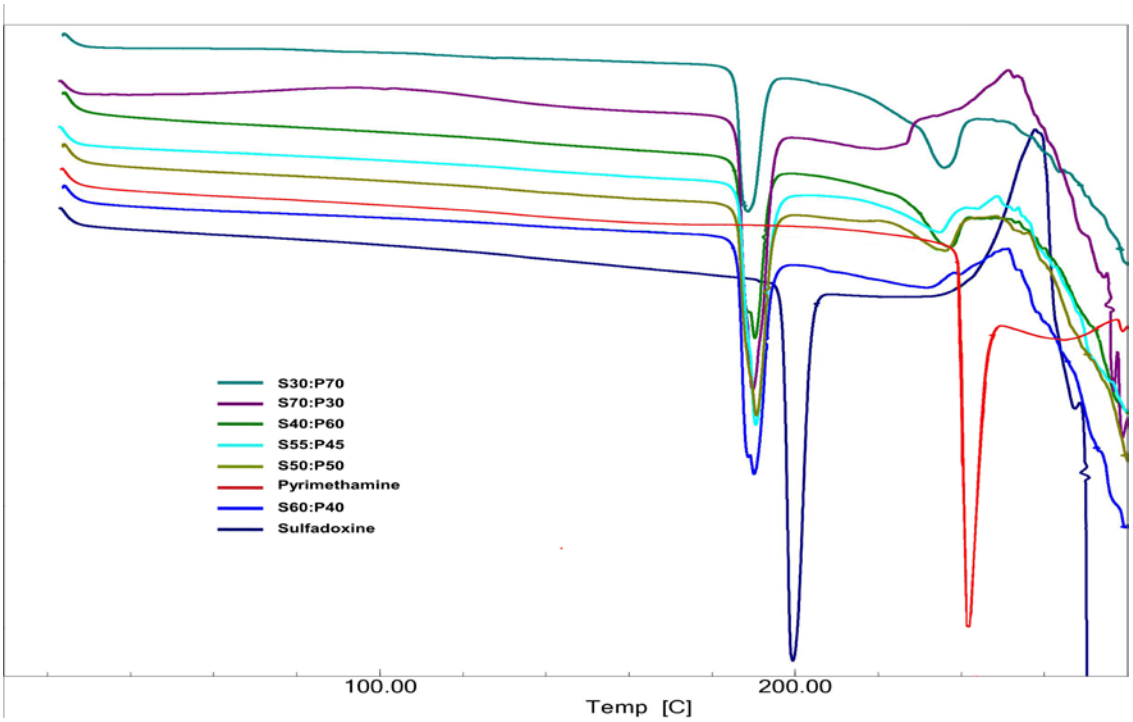


**Figure 4.36:** Overlay of the DSC thermograms of the two single components and combinations at different % w/w ratios.

### 4.5.1 Discussion

The melting temperature for sulfadoxine is indicated as 199.43°C (Figure 4.28) and that for pyrimethamine as 243.24°C (Figure 4.29). These obtained results correlate with the melting temperatures indicated in the BP (2016) of 198°C for sulfadoxine and 239 - 242°C for pyrimethamine. A difference of 1 - 2°C as seen with pyrimethamine is still within limits in terms of thermal analysis, since thermal events greatly depend on the heating rate that was used during the analysis. In Figures 4.30 and 4.31 only one melting endotherm is seen for the ratios S70:P30 (189.79°C) and S60:P40 (190.07°C). On the other hand the ratios of S55:P45, S50:P50 , S40:P60 and S30:P70 all showed two melting endotherms, one signifying the melting of sulfadoxine and one showing the melting temperature of pyrimethamine (Figures 4.32 – 4.35). A summary of the thermal events are given in table 4.42.

**Table 4.42:** Summary of the thermal events obtained during this study

| Sample | Sulfadoxine melting point (°C) | Heat of fusion (J/g) | Pyrimethamine melting point (°C) | Heat of fusion (J/g) |
|---|---|---|---|---|
| Sulfadoxine single component | 199.43 | 117.02 | - | - |
| Pyrimethamine single component | - | - | 243.24 | 534.75 |
| S70:P30 | 189.79 | 107.49 | - | - |
| S60:P40 | 190.07 | 94.26 | - | - |
| S55:P45 | 190.52 | 79.03 | 234.80 | 21.56 |
| S50:P50 | 190.65 | 78.72 | 236.13 | 23.04 |
| S40:P60 | 190.30 | 64.35 | 237.37 | 23.25 |

The single endotherm, in the presence of two components (Figures 4.30 – 4.31) might be a result of the specific weight of pyrimethamine in combination with sulfadoxine. If pyrimethamine has a weight less than sulfadoxine in the combination, only one endotherm is present and this could be due to the fact that pyrimethamine either dissolves in or interacts with the sulfadoxine and therefore forming only one endotherm. A eutectic mixture is being defined by the Oxford Dictionary of Chemistry (2008), as: “A solid solution consisting of two or more substances and having the freezing point of any possible mixture of these components. The minimum freezing point for a set of components is called the

eutectic point". In pharmaceutical terms the freezing point can also be the melting point. According to several other literature reports, eutectic mixtures form when a molten state containing both compounds either crystallises to form very fine, microcrystals of the two components or if the crystallisation of one of the components is being inhibited (Gala *et al*., 2013: 1, Sekiguchi and Obi, 1961: 866; Serajuddin, 1999: 1058; Stott *et al*., 1998: 298).

In Figures 4.32 - 4.35 two melting endotherms are present and this is possibly due to the fact that there is more pyrimethamine in the combination than sulfadoxine. As seen from the presented results, pyrimethamine melts at a higher temperature than sulfadoxine, therefore no or only partial solubilisation of pyrimethamine in the lower but melted sulfadoxine concentration occurs.

### 4.5.2 Conclusion

Results indicate that in certain ratios of the SP combination the two actives influence each other in such a way that only one melting endotherm is formed, indicative of the formation of a possible eutectic mixture with a melting temperature lower than that of both the single components.

## 4.6 X-Ray Powder Diffraction (XRPD)

The following graphs indicate the XRPD results:

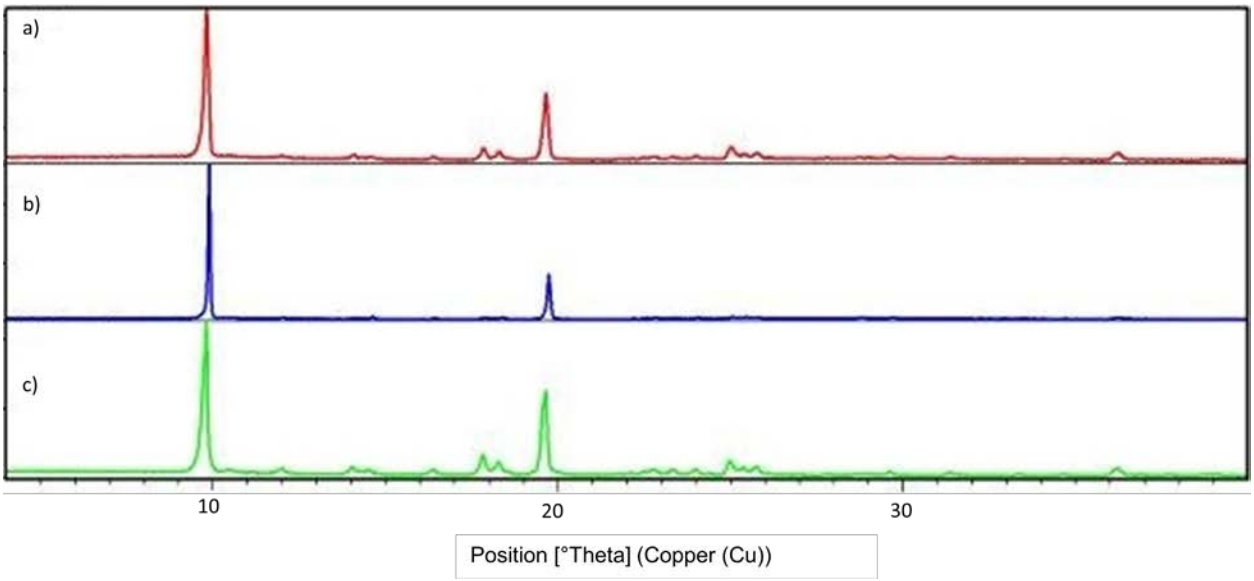


**Figure 4.37:** XRPD patterns for a) sulfadoxine single compound, b) sulfadoxine single compound after an hour in distilled water and c) sulfadoxine single compound after eight hours in distilled water.

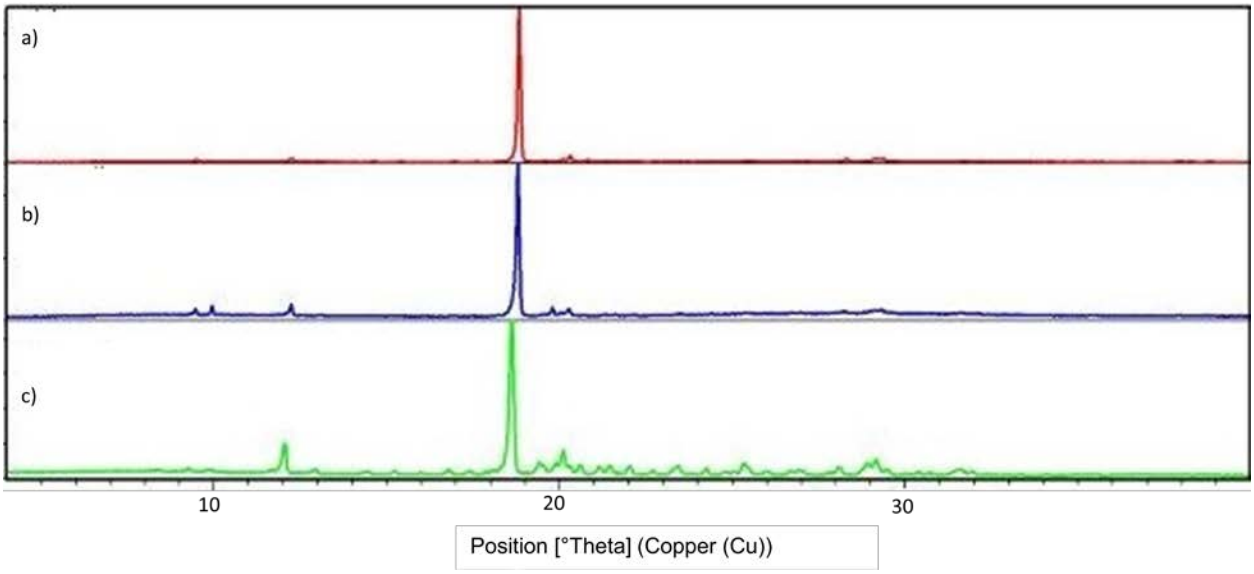


**Figure 4.38:** XRPD patterns for a) pyrimethamine single compound, b) pyrimethamine single compound after an hour in distilled water and c) pyrimethamine after eight hours in distilled water.

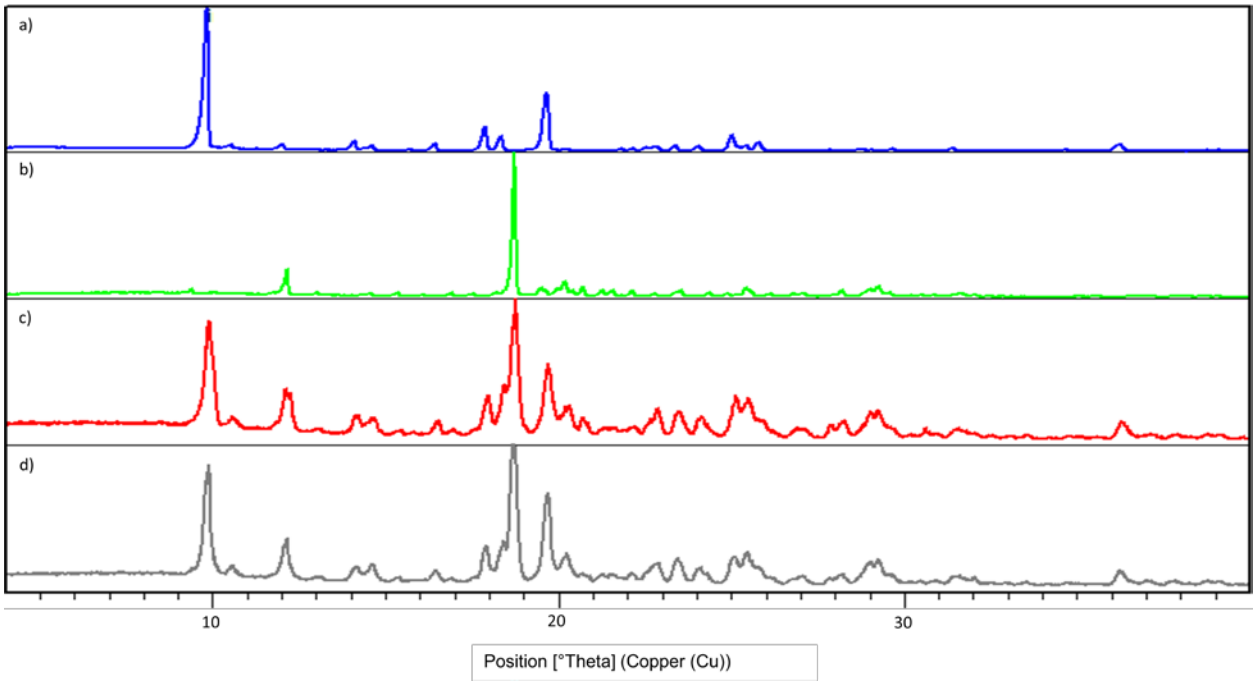


**Figure 4.39:** XRPD patterns for a) sulfadoxine single compound, b) pyrimethamine single compound, c) S50:P50 without distilled water and (d) S55:P45 without distilled water.

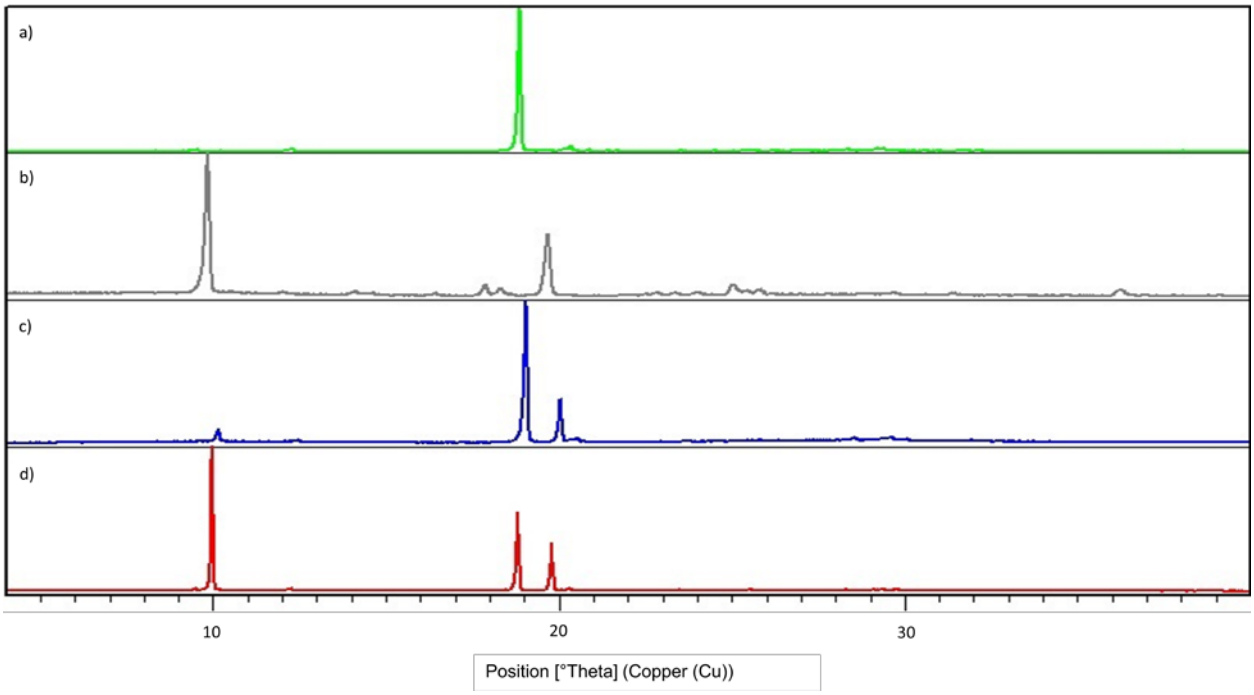


**Figure 4.40:** XRPD patterns for a) pyrimethamine single compound, b) sulfadoxine single compound, c) S50:P50 with distilled water and (d) S55:P45 with distilled water.

### 4.6.1 Discussion

Figures 4.37 and 4.38 depict the XRPD results of sulfadoxine and pyrimethamine as single compounds. It also indicated the results after one hour and after eight hours. None of the diffraction peaks shifted and no new diffraction peaks (after eight hours) in both single components has formed.

Figures 4.39 and 4.40 indicate the single components and SP combinations with and without water. Comparing the SP combinations to the single components, no shifting or formation of new diffraction peaks is present.

### 4.6.2 Conclusion

The results emphasised that there were no new formation or shifting of the diffraction peaks and therefore indicating that no new crystal forms or even co-crystals of these two actives formed.

## 4.7 References

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USP ***see*** United States Pharmacopoeia

# CHAPTER 5

## SUMMARY AND CONCLUSION

Prevention of malaria is of great importance in pregnant women as they are more prone to be infected with malaria due to their hormonal changes. Intermittent preventive therapy such as sulfadoxine in combination with pyrimethamine is still recommended by the WHO; but the increase of resistance to these two drugs is becoming a major concern (Rogawski *et al*., 2012:1096, Wells *et al.*, 2009:879).

Poor solubility and dissolution rate of both of these drugs remain a challenge during development. This results in poor therapeutic efficiency which compromises the drug prophylaxis and cause gastrointestinal mucosa toxicity. Furthermore, the exposure of sub-lethal SP concentrations, to the malaria parasite, will result in an elevated occurrence of drug resistance (Sinnaeve *et al*., 2005:97).

The mentioned problem of very poor solubility and slow dissolution rates of SP must be evaluated and considered critically. These two very important properties of the two drugs might actually trigger the development of parasitic resistance. Addressing these two important properties and re-thinking the way that they are formulated might combat malaria infection and the resistance of the malaria parasite to preventive therapy.

Aims and objective were set out to investigate the physico-chemical, solubility and dissolution properties of sulfadoxine and pyrimethamine in different combinations.

Preparation of the sulfadoxine and pyrimethamine combinations and capsulating these mixtures into hard gelatine shells were done successfully. After preparation and encapsuling of the SP mixtures the melting point and structural characteristics of sulfadoxine and pyrimethamine were determined by the use of DSC and XRPD.

The XRPD results of sulfadoxine and pyrimethamine as single components and in different ratios indicates that there is no shifting or formation of new diffraction peaks. These results emphasised that there were no recrystallization of these two actives to form a hydrate or any other solid state.

The solubility and dissolution rate of the SP combinations were done in water, PBS pH 6.8 and 0.1 N HCl. The solubility results emphasised that sulfadoxine and pyrimethamine is the best soluble in 0.1 N HCl with concentrations of 1190 µg/ml and 2714 µg/ml respectively. The lowest

solubility results obtained was 244 µg/ml and 34 µg for sulfadoxine and pyrimethamine respectively in distilled water.

The S500:P25 combination performed the best in 0.1 N HCl for sulfadoxine and pyrimethamine with concentrations of 1192 µg/ml and 972 µg/ml, respectively. This could be to the fact that there as less pyrimethamine available in this specific combination.

Comparative results between the single components and SP combinations indicated that when sulfadoxine is in combination with pyrimethamine the solubility of sulfadoxine increases in both water and PBS. There was no fixed pattern in both of the media but the increase of sulfadoxine was more significant in water than in PBS. The comparative results between pyrimethamine as single component and the SP combinations indicated that there is a slight increase in the solubility of pyrimethamine when in combination with sulfadoxine in water and PBS.

Comparing the 0.1 N HCl results with water and PBS the effect of the solubility of sulfadoxine and pyrimethamine changes completely. When the two actives are in combination with each other, the solubility of both sulfadoxine and pyrimethamine decreases significantly. Dissolution studies were performed in water, PBS (pH 6.8) and 0.1 N HCl. In only a few instances the USP requirements for the FDC have been met but should however be noted that these requirements are for drug formulations and not for powder dissolutions. A 60% dissolution percentage or higher in 30 minutes for sulfadoxine were observed in only three instances in water: sulfadoxine as single component (60.5%), S500:P25 (63.0%) and S55:P45 (63.2%) combination. For pyrimethamine, the S500:P25 (74.6%) was the only combination to reach a dissolution percentage of 60% or higher. A dissolution percentage of 60% or higher after 30 minutes in PBS for sulfadoxine was reached: as single component (87.0%), S500:P25 (92.0%), S50:P50 (66.4%) and S55:P45 (65%.0). For pyrimethamine, the single component nor the SP combination reached a dissolution percentage of 60% or higher in 30 minutes in PBS.

A 60% dissolution percentage or higher in 30 minutes for sulfadoxine were observed in only five instances in 0.1 N HCl: single component (86.1%), S40:P60 (62.5%), S50:P50 (60.4%), S60:P40 (70.0%) and S70:P30. For pyrimethamine, five combinations reached a dissolution percentage of 60% or higher in 0.1 N HCl.

The results of the DSC indicated that the melting temperatures for sulfadoxine (199.34°C) and pyrimethamine (243.24°C) correlates with the melting temperatures indicated in the BP (198°C for sulfadoxine and 239 - 242°C for pyrimethamine) (BP, 2016). For ratios S70:P30 (189.79°C) and

S60:P40 only one endotherm has formed. This phenomenon could be due to the fact that small amounts of pyrimethamine melt in sulfadoxine and thus only one endotherm is seen. At certain ratios sulfadoxine and pyrimethamine influence each other to form only one endotherm which is indicative of the formation of a eutectic mixture with melting temperature lower than both of the single components (Habib, 2001:17).

Two clear endotherms for ratios S40:P60 (sulfadoxine – 190.30°C and pyrimethamine – 237.37°C), S50:P50 (sulfadoxine - 190.65°C and pyrimethamine – 236.13°C), S55:P45 (sulfadoxine - 190.52°C and pyrimethamine – 234.80°C) and S70:P30 (sulfadoxine - 188.56°C and pyrimethamine 235.91°C) were seen and indicates that with larger amounts of pyrimethamine, in relation to sulfadoxine, pyrimethamine does not completely melt in sulfadoxine.

Information regarding the physico-chemical, solubility and dissolution properties of sulfadoxine and pyrimethamine as single compounds and sulfadoxine and pyrimethamine combinations were collected during this study. The results indicated the possibility of the formation of a eutectic mixture and further studies should be conducted to confirm this possibility.

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