

Physico-chemical characterisation and compatibility of selected anti-malarial drugs

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**In profound gratitude and loving memory of my beloved grandfather, J.J.
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Trust in the Lord with all your heart and lean not on your own understanding; in all your ways submit to Him, and He will make your paths straight.

-Proverbs 3:5 – 6

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ABSTRACT

Malaria is an extensive, potentially fatal disease caused by the *Plasmodium* parasite, which is transmitted to humans by female *Anopheles* mosquitoes. One specific problem is the growing resistance of the parasites to active pharmaceutical ingredients (APIs). Thus, sulfadoxine, pyrimethamine, and artesunate are available as a comb-pack kit. When combination therapy is taken orally, the APIs may influence one another's physico-chemical properties, such as solubility.

Compatibility studies are usually performed between APIs and their excipients and, to a lesser extent, between different APIs indicated for the same disease or administered in an FDC. It is imperative to thoroughly investigate the compatibility of APIs to ensure that a formulation of the different components is complementary to one another. Physico-chemical characterisation studies were performed to provide insight regarding compatibility and possible influences on solubility. The study used thermal activity microcalorimetry (TAM) to investigate the compatibility of each API, individually and in combination, and found no observable interactions. Additionally, differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) spectroscopy were performed, proving the dispersion of one API into another.

Solubility studies of sulfadoxine, pyrimethamine, and artesunate were investigated (separately and in combination) in various media over a pH range of 1.2 – 6.8. Artesunate displayed degradation in various media, except in one buffer media at pH 4.5. Additionally, a pH-dependent trend was seen with the highest solubility in potassium phosphate (PBS) buffer.

Pyrimethamine presented higher solubilities at a lower pH and poor solubility in higher pH ranges. The solubility of pyrimethamine was significantly influenced by the presence of both artesunate and sulfadoxine, respectively, with the best improvement in the artesunate-pyrimethamine combination in water. In contrast, sulfadoxine exhibited improved solubility only in the acetate (ACE) buffer and water when combined with pyrimethamine.

Key words: Artesunate, pyrimethamine, sulfadoxine, solubility, compatibility studies, physico-chemical characterisation.

Table of Contents

CHAPTER 1: INTRODUCTION	1
1.1. Introduction	1
1.2. Research problem	2
1.3. Aim and objectives	2
1.4. Chapter layout of dissertation	2
References	4
CHAPTER 2: LITERATURE OVERVIEW	5
2.1. Malaria	5
2.1.1. Parasites	6
2.1.2. Pathogenesis	6
2.1.3. Clinical presentation and symptoms	8
2.1.4. Diagnosis	9
2.1.5. Prevention and treatment	9
2.2. Active pharmaceutical ingredients	11
2.2.1. Artesunate	11
2.2.2. Pyrimethamine	14
2.2.3. Sulfadoxine	16
2.3. Biopharmaceutical Classification System (BCS)	18
2.4. Active pharmaceutical ingredient solubility	20
2.4.1. Temperature and heat input	21

2.4.2.	Molecular structure of the solute.....	21
2.4.3.	Nature of solvent	21
2.4.4.	Particle size.....	22
2.4.5.	pH	22
2.4.6.	Solid–state properties.....	24
2.5.	Compatibility of active pharmaceutical ingredients	24
2.6.	Summary.....	25
References		27
CHAPTER 3: MATERIALS AND METHODS.....		37
3.1	Introduction	37
3.2.	Materials	38
3.3.	Research Design.....	38
3.4.	Methods	39
3.4.1.	High-performance liquid chromatography (HPLC)	39
3.4.2.	Solubility studies.....	44
3.4.3.	Buffers and solution preparation.....	46
3.4.4.	Statistical analysis	47
3.4.5.	Physico-chemical characterisation	48
References		51
CHAPTER 4: RESULTS AND DISCUSSION		54
4.1	Introduction	54

4.2	HPLC method validation.....	54
4.2.1	Artesunate method validation in different buffer solutions.....	54
4.2.2	Pyrimethamine method validation in different buffer solutions	56
4.2.3	Sulfadoxine method validation in different buffer solutions	58
4.3	Solubility studies.....	60
4.3.1	Artesunate.....	60
4.3.2	Pyrimethamine	82
4.3.3	Sulfadoxine	112
4.4	Statistical analysis.....	132
4.4.1	ANCOVA analysis of preliminary studies.....	132
4.4.2	ANOVA analysis for pivot studies	136
4.5	Physico-chemical studies	138
4.5.1	Differential scanning calorimetry (DSC).....	138
4.5.2	Fourier transform infrared spectroscopy (FT-IR).....	140
4.5.3	Thermal Activity Microcalorimetry (TAM)	143
4.6	Conclusion	144
References		148
CHAPTER 5: CONCLUSION.....		150
5.1	Conclusion	150
5.2	Future recommendations.....	152
References		152

ANNEXURE A: SPECIFICITY ON ARTESUNATE METHOD.....	153
ANNEXURE B: SPECIFICITY ON SULFADOXINE/PYRIMETHAMINE METHOD.....	157

CHAPTER 1: INTRODUCTION

1.1. Introduction

Plasmodium falciparum is one of five single-cell parasites mainly responsible for causing the most incidences of malaria in humans ([Rosenthal, 2018](#)). Globally, malaria cases increased by approximately 2 million from 2020 to 2021 in 84 countries. This remains an enormous problem, especially since the year 2020 was associated with the disruption of services during the coronavirus disease (COVID-19) outbreak ([WHO, 2022](#)).

The majority of malaria cases are concentrated in the African region, which accounts for 95 % of all reported cases. From 2019 to 2020, an increase from 218 million to 232 million cases were reported, while mortality during the same time increased by approximately 55 000. The total fatality rate, however, decreased in 2021 to 593 000, of which 78.9 % were children under 5 years of age ([WHO, 2022](#)).

Increased resistance to malaria treatment in the African region is a real concern. Pyrimethamine-sulfadoxine combination therapy was used as a replacement therapy to chloroquine, but resistance rapidly developed in East Africa. This had a negative impact on childhood mortality ([Von Seidlein et al., 2000](#)).

As a result, artesunate (an artemisinin-derivate) in combination with sulfadoxine and pyrimethamine was introduced as a fixed dose combination therapy for uncomplicated *Plasmodium falciparum* by the WHO in the African Region ([WHO, 2022](#)). Artemisinin and its derivatives, including artesunate, can decrease the parasite load rapidly, but unfortunately, has a short half-life. Pyrimethamine-sulfadoxine is longer-acting, which is beneficial to prevent artemisinin-resistant parasites. In areas where partial resistance to pyrimethamine-sulfadoxine exists, this combination prevents the further spread of resistance ([Von Seidlein et al., 2000](#)).

Drug-drug compatibility is a major consideration in combination therapy as it may impact not only pre-formulation but also manufacturing and administration of drugs, even more so in the treatment of malaria due to the parasite's resistance to monotherapy. Additionally, the oral administration of multiple APIs during treatment may negatively affect patient compliance ([Jeličić et al., 2021](#)). Upon the combination of APIs in a fixed dose combination, it is important

to guarantee bioavailability, product stability, as well as safety by assessing the interactions the various components may exert on each other ([Veiga et al., 2017](#)). APIs differ from each other in terms of their properties, such as crystalline structure, pH, solubility, dissolution rate, and bioavailability. When combining two or more APIs, these properties may be altered, resulting in either conflicting or complementary interactions. When these interactions are conflicting (less desirable), it indicates incompatibility ([Patel et al., 2015](#)), possibly leading to a dormant or inactive API, which in turn results in a decrease in safety and efficacy ([Dourado, 2019](#)).

1.2. Research problem

Malaria, more specifically *P. falciparum* infection, remains a global problem especially in the African region. The prevalence of API resistance provides various challenges to the prevention and treatment of the disease. API combination therapy such as artesunate, sulfadoxine and pyrimethamine have been introduced to aid in the successful treatment of *P. falciparum* malaria. It is of importance to investigate these APIs in combination with regard to the influence they might exert on each other's solubility and compatibility.

1.3. Aim and objectives

The aim of this study is to establish whether any of the three APIs has an influence on each other's solubility and to establish the compatibility of these APIs using physico-chemical characterisation. To achieve the aim of this study the following objectives were set:

- Verify existing high-performance liquid chromatography (HPLC) methods for all three APIs, in-house.
- Perform solubility studies of all three APIs (individually and in combination) in various media, over a pH range of 1.2 to 6.8.
- Perform compatibility studies of all three APIs (individually and in combination) using thermal activity monitoring (TAM).
- Perform physico-chemical characterisation of all three APIs (individually and in combination) using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR).

1.4. Chapter layout of dissertation

This dissertation consists of five chapters. Chapter 1 provides an introduction together with the research problem, aim and objectives. In chapter 2, a thorough literature review is presented

whilst in chapter 3 all methods used during this study are discussed. Chapter 4 conveys all results obtained throughout this study, together with a discussion on all findings. Finally, in chapter 5 conclusions and future prospects are provided.

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CHAPTER 2: LITERATURE OVERVIEW

2.1. Malaria

Malaria is an extensive, potentially fatal disease caused by protozoan parasites that get transmitted by the bite of female *Anopheles* mosquitoes. Evidence of diseases resembling malaria can be found in historical texts, including biblical passages and writings by *Hippocrates*, dating back to ancient times ([Boualam et al., 2021](#)). The term “malaria” originated from the Italian word “mal’aria” which meant “bad air” during the Middle Ages ([Nájera-Morrondo, 1991](#); [Boualam et al., 2021](#)). During the late 1800s, the presence of parasites in patients’ blood was discovered by physician Charles Louis Alphonse Laveran ([Manson-Bahr, 1961](#); [Schlagenhauf, 2004](#)). In 1897, Ronald Ross made a significant breakthrough by connecting *Anopheles* mosquitoes and the transmission of malaria parasites to humans ([Rieckmann, 2006](#); [Manson-Bahr, 1961](#)).

Prior to the 1940s, malaria affected around two-thirds of the world’s population ([Ingraham & Ingraham, 2004](#)). In 1955, the World Health Organization (WHO) initiated eradication programs ([WHO, 1957](#)). Initially, control measures were implemented to reduce mosquito populations and, consequently, interrupt transmission. Measures included the use of bed nets, draining swamps, and dichlorodiphenyltrichloroethane (DDT), an insecticide ([Motohira et al., 2019](#); [Nájera-Morrondo, 1991](#)). Malaria became manageable between the 1950s and 1960s, particularly in temperate regions where the disease was mostly eradicated ([Ingraham & Ingraham, 2004](#)).

As a result, research funds were cut and anti-malarial programs were scaled back. However, a major setback occurred in the 1970s when mosquitoes became DDT-resistant. Additionally, the *Plasmodium falciparum* parasite became drug-resistant, resulting in the ongoing prevalence of malaria ([Ingraham & Ingraham, 2004](#)).

Globally, the number of malaria cases has risen by approximately 2 million from 2020 to 2021, reaching a total of 247 million cases across 84 countries. This significant increase is concerning, especially considering the impact of the COVID-19 pandemic on these numbers. Prior to the pandemic, there was a consistent decrease in malaria cases, but since 2020, the rise can be attributed to disruptions caused by COVID-19 ([WHO, 2022](#)).

The African region bears the brunt of malaria cases, accounting 95 % of the total reported cases. From 2019 to 2020, the number of cases increased from 218 million to 232 million, while the mortality rate also increased from 544 000 to 599 000. However, in 2021, the number of deaths decreased to 593 000. Within the African region, these figures represent 96 % of global malaria-related deaths. Notably, 78.9 % of the deaths in the African region are children under the age of 5, compared to 91 % in the year 2000 ([WHO, 2022](#)).

2.1.1. Parasites

Of the 400 different *Anopheles* species in the world, 60 female *Anopheles* mosquito species have malaria vectors infecting humans ([Paniker, 2018](#)). Six species of the *Plasmodium* parasites are responsible for malaria disease in humans: *Plasmodium falciparum* (*P. falciparum*), *P. malariae*, *P. vivax*, *P. ovale wallickeri*, *P. ovale curtisi*, and recently *P. knowlesi* ([Milner, 2018](#)). *P. falciparum* is mainly responsible for causing the majority of malaria cases and deaths in humans ([Rosenthal, 2018](#); [Cowman et al., 2016](#)). These parasites are eukaryotic single-celled micro-organisms which differs geographically, immunologically, morphologically, in drug responses and, relapse patterns ([Tuteja, 2007](#)). Malaria is mostly common in tropical and sub-tropical areas, where it is also difficult to control or eradicate. Two most widespread malaria over the malaria belt are *P. falciparum* and *P. vivax*, however *P. vivax* is not found in West Africa. This is due to the fact that the malaria species penetrate Duffy buffer blood groups and most of the human population in West Africa don't have this in their red blood cells. Two more uncommon species are *P. ovale* and *P. malariae*. *P. ovale* is second ranked in West Africa after *P. falciparum*. *P. malariae* is widespread even though it is uncommon, and its effects are relatively benign ([Gunn & Pitt, 2012](#); [Brooks et al., 2013](#)). *P. vivax* and *P. ovale* are capable of causing relapse patterns, where *P. falciparum* and *P. malariae* invade the liver with only one cycle and the liver infection ceases within four weeks ([Rosenthal, 2018](#)).

2.1.2. Pathogenesis

2.1.2.1. Mosquito-human life cycle of malaria

The life cycle of the malaria parasites (displayed in Figure 2.1) can be perceived as complex presenting with various stages/cycles. In order for the parasites to survive, the cycle alternates between two hosts, vertebrate and invertebrate. Proteins inside the host are needed for synthesis and the survival of the parasites, to invade different cell types and evade the host's immune responses. This can occur intracellularly and extracellularly ([Bray & Garnham, 1982](#)).

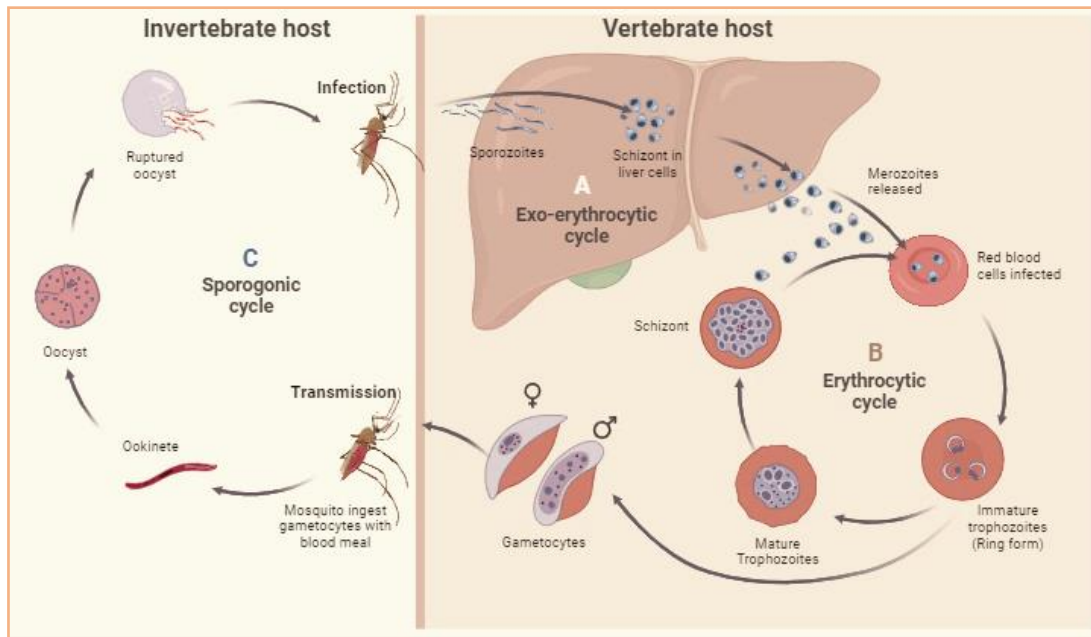


Figure 2.1: Adapted version of the malaria life cycle ([CDC, 2022](#); [BioRender, 2023](#)).

Exo-erythrocytic cycle. During this cycle female infected *Anopheles* mosquitoes injects saliva, containing infective sporozoites (*Plasmodia* parasites) and anticoagulants (ensuring an easy ingestion) into the skin of the host. The sporozoites move rapidly into the bloodstream and reach the liver. This usually occurs more or less in an hour. The sporozoites then enters the liver hepatocytes. The first phase of asexual reproduction starts and the cells differentiate either in schizonts or hypnozoites. This process is called, exo-erythrocytic schizogony. These exo-erythrocytic schizonts mature when the nucleus divides, and when the cytoplasmic volume increase. Maturing results in forming uninucleate merozoites. At maturity, the schizonts bursts and the merozoites are released into the bloodstream ([Paniker, 2018](#); [Ingraham & Ingraham, 2004](#)). Hypnozoites (usually created by the *P. vivax* and *P. ovale* parasites) remain in the liver for longer periods of time, before development, causing relapses in the human body ([Gunn & Pitt, 2012](#)).

Erythrocytic cycle. The second phase of asexual reproduction starts when the merozoites invade red blood cells (erythrocytes). This happens within minutes, because the merozoites are short-lived. Upon entry into the erythrocyte, the merozoite develops a vacuole and changes into a ring form, with the nucleus as the seal. The ring form grows and increases in cytoplasmic volume until the vacuole disappears. This is now called a trophozoite, and feeds on erythrocytic cytoplasm. At the end of the trophic stage, multiple nuclear division result in formation of

schizonts and production of merozoites. Schizonts mature, disintegrate and the merozoites are released. This process remains in a cycle, resulting in an accretion of parasites that manifests as the disease known as malaria ([Paniker, 2018](#); [Gunn & Pitt, 2012](#)).

Gametogony. Several of the merozoites enters the sexual phase, growing in size until the erythrocyte is almost filled, but the nucleus remains undivided. The exact stimulus is unknown. Young merozoites mature into larger female (macrogametocyte) or smaller male (microgametocyte) gametocytes. These gametocytes circulate until the female *anopheline* mosquito feeds and uptake the gametocytes ([Paniker, 2018](#); [Brooks et al., 2013](#)).

Sporogonic cycle. The gametocytes develop into infective sporozoites whilst inside the mosquito ([Rosenthal, 2018](#)). After ingestion, the female macrogametocytes and the male microgametocytes gather in the mid-gut of the mosquito. The microgametocyte divides and undergoes maturation becoming a microgamete. The macrogametocytes only undergoes a process of maturation, before breaking free from erythrocyte becoming macrogamete. The latter is fertilised by one of the microgametes to produce a zygote. Zygotes are initially motionless, but gradually elongates becoming a travelling vermicle (ookinete). The ookinete permeates through the mosquito's stomach wall, becoming an oocyst multiplying to form sporozoites. The sporozoites then migrate to the salivary glands of the mosquito to be injected into humans whilst the mosquito feeds ([Paniker, 2018](#)).

2.1.3. Clinical presentation and symptoms

Simultaneously, with the malaria life-cycle (as described above), the human body's immune system release macrophages and cytokines as a response, presenting with clinical symptoms. With the initial acute febrile attacks, the following symptoms occur, lasting in total about six to ten hours ([CDC, 2022](#); [Paniker, 2018](#)). These acute attacks can be divided into three stages:

Cold stage. It occurs first and continues for about 15 min to 1 h. Symptoms include sensations of intense cold, together with chills. Headaches and nausea also transpire ([CDC, 2022](#); [Paniker, 2018](#)).

Hot stage. This is the following stage, after the cold stage. It persists for approximately 2 – 6 h. Symptoms include fever, with body temperatures reaching 41°C or higher. Sometimes seizures in young children are seen. Headaches last during this stage, while nausea tends to reduce ([CDC, 2022](#); [Paniker, 2018](#)).

Sweat stage. Now, the body undergoes profuse sweating as it tries to cool down after the hot stage. The skin can feel moist and cooled ([CDC, 2022](#); [Paniker, 2018](#)).

Without proper diagnosis and treatment, the illness quickly progresses to the severe form of the disease. Severe malaria is considered to result in severe anaemia and haemoglobinuria (the presence of haemoglobin in the urine) caused by haemolysis, acute respiratory distress syndrome, metabolic acidosis, and cerebral malaria. Depending on the area, people tend to develop partial immunity against malaria parasites, leading to asymptomatic infections ([CDC, 2022](#); [Paniker, 2018](#)).

2.1.4. Diagnosis

The accurate and timely diagnosis of malaria is significant as it enables effective early treatment to manage and control this life-threatening disease while preventing serious complications and mortalities. The approach to diagnose malaria has to be comprehensive, which can include a variety of diagnostics methods. Healthcare professionals sometimes employ more than one method to ensure a thorough assessment, which can include, patient history, laboratory tests, and clinical consults over a time-frame to confirm the presence of malaria ([Tuteja, 2007](#)).

Laboratory tests are generally employed as the primary diagnostic system – the “gold standard.” It involves examining thick and thin blood smears under a microscope, enabling the identification and quantification of the malaria parasites, specifically, the type of species. Even though the tests are accurate, it is not always available to everyone and necessitates the experience of a trained professional. Alternately, rapid antigen discovery tests (RDTs) offer a quick and user-friendly option. These tests descry parasite proteins through a small finger-prick and can deliver results within minutes. However, RDTs are limited to identifying certain parasite species like, *P. falciparum* and *P. vivax*, and incapable to quantify the parasite load. RDTs are most effective when the temperature of the human is rising and the parasite count is at its highest. Another approach is the polymerase chain reaction (PCR), which is very sensitive and able to detect and identify parasites in low quantities through DNA extraction ([Suh et al., 2004](#); [Crutcher & Hoffman, 1996](#)).

2.1.5. Prevention and treatment

Since mosquitoes had been identified as the vectors, attempts to control the disease, have been implemented. Attempts include, treating human infections, and reducing the transmission

([Crutcher & Hoffman, 1996](#)). The WHO compiled an essential medicine list, as seen in Table 2.1 with preferred treatment guidelines and various fixed-dose combinations (FDCs) for the chemoprevention and curative treatment of malaria ([WHO, 2021](#)). Malaria transmission can be alleviated through several strategies. One approach includes decreasing the mosquito population, by shortening its life-span in order to interrupt the malaria parasite's development. Shortened life-span can include; elimination of breeding sites by draining swamps, insecticide-treated bed nets, and application of residual insecticides like DDTs ([Crutcher & Hoffman, 1996](#); [Suh et al., 2004](#)).

Table 2.1: Treatment guideline for curative and chemoprevention of malaria ([WHO, 2021](#)).

API	Curative	Preventative	Combination therapy
Amodiaquine	✓	✓	Amodiaquine + artesunate
Artemether	✓	-	Artemether + lumefantrine
Artesunate	✓	-	Artesunate + mefloquine Artesunate + sulfadoxine + pyrimethamine
Chloroquine	✓	✓	Chloroquine + proguanil
Dihydroartemisinin	✓	-	Dihydroartemisinin + piperaquine phosphate
Doxycycline	✓	✓	Doxycycline + quinine
Lumefantrine	✓	-	
Mefloquine	✓	✓	
Piperaquine phosphate	✓	-	
Primaquine	✓	-	
Proguanil	-	✓	
Pyrimethamine	✓	✓	Amodiaquine + pyrimethamine +sulfadoxine
Quinine	✓	-	
Sulfadoxine	✓	✓	

Artesunate is used in the asexual blood stage of the malarial life-cycle. By rapidly clearing the parasites from the blood, these APIs interrupt the replication and spread of the parasite,

effectively breaking the malarial life-cycle and preventing further damage to the host's red blood cells. This action not only helps alleviate the acute symptoms but also reduces the potential for complications and transmission to other individuals through mosquito bites ([Rosenthal, 2008](#)).

Additionally, sulfadoxine and pyrimethamine are synergistic in action. Both are used to act during the asexual blood stage of the malarial cycle. By interfering with folate synthesis, sulfadoxine and pyrimethamine disrupts the production of essential nucleic acids (DNA and RNA) required for the malaria parasite's survival and reproduction during the asexual blood stage ([Shapiro & Goldberg, 2006](#)).

The reasoning behind combination therapy is to address the growing problem of API resistance, which has been observed with single-drug treatments. Pyrimethamine and sulfadoxine resistance are a real concern. Over time, the *P. falciparum* parasite mutated to survive the treatment options for malaria. Mutations on the enzymes responsible for folic acid production, decrease the affinity for APIs to bind to the enzyme structure. These mutations are a key factor in sulfadoxine-pyrimethamine treatment failure ([Beauduy & Winston, 2018](#); [Kublin, et al., 2002](#)). Additional factors to increase resistance, are incorrect dosing, non-compliance with duration of dosing regimen, misdiagnosis, drug interactions, poor absorption and API quality ([Bloland, 2001](#)). By using two or more APIs with different modes of action, will minimise the likelihood of resistance development, ensuring the continued efficacy of the treatment. For instance, adding artesunate (short acting anti-malarial) to the combination sulfadoxine-pyrimethamine (long acting anti-malarial) ([Rosenthal, 2008](#)).

2.2. Active pharmaceutical ingredients

Artesunate, pyrimethamine and sulfadoxine are used in a fixed dose combination available as a combination pack kit containing 50 mg of artesunate, 25 mg pyrimethamine and 500 mg sulfadoxine ([WHO, 2021](#)).

2.2.1. Artesunate

Artemisinin is a natural derivative known as a sesquiterpene lactone, which is extracted from a Chinese herb called Qinghao or *Artemisia annua* ([Chinaeke et al., 2014](#)). Qinghao has been traditionally used since ancient times to alleviate fever and chills. In 1971, the medicinal plant was rediscovered and anti-malarial properties investigated, leading to the identification of artemisinin (also known as Qinghaosu). In 1979, more potent derivatives that were soluble in

both oil and water were developed to overcome challenges with bioavailability ([Su & Miller, 2015](#)).

Initially, Qinghaosu and its derivatives, including dihydroartemisinin, artemether, artheether, and artesunate, were used in China exclusively. However, due to the increasing prevalence of mutated *P. falciparum* strains, the World Health Organization (WHO) included these derivatives as a first-choice treatment globally, as an attempt against the drug resistance ([Chang, 2015](#); [White, 2008](#)).

Among the derivatives, artesunate, also referred to as dihydroartemisinin-12- α -succinate, is the most extensively studied compound. It is a semi-synthetic compound developed through a two-step reaction involving reduction and esterification ([Al Haydar, 2011](#)).

2.2.1.1. Pharmacology

Artesunate possesses a broad spectrum of activity. Its lipophilic nature allows it to easily cross erythrocyte and parasite membranes ([Al Haydar, 2011](#)). Artesunate triggers an oxidative process through the presence of an endoperoxide bridge on the 1,2,4-trioxane ring, which interacts with ferrous heme. The parasites produce heme during the degradation of haemoglobin. Consequently, artesunate induces the cleavage of the bridge through a reaction with ferrous iron. This metabolic stress leads to the release of highly reactive free radicals, including carbon centered and artemisinin-derived radicals. These radicals covalently bind to the parasite's membrane proteins, initiating lipid peroxidation. As a result, the altered membrane hampers nutrient supply to the parasite, causing damage to the endoplasmic reticulum in intra-erythrocytic plasmodia. Ultimately, this process leads to cell death and lysis of the parasite ([Raffetin, et al., 2018](#); [Barradell & Fitton, 1995](#); [Adebayo et al., 2020](#)).

Artesunate is used for the treatment of uncomplicated and severe malaria. In addition to its anti-malarial properties, artesunate has demonstrated other anti-infective actions, including anti-parasitic activity against schistosomiasis, as well as anti-viral, antifungal, and antibacterial activities ([Li & Weina, 2010](#); [Raffetin et al., 2018](#)).

Moreover, artesunate has shown potential non-infectious mechanisms for treating tumours, inflammation and haemorrhages. Notably, these indications have only been tested in animal studies, as highlighted by [Raffetin et al., 2018](#). Therefore, further data and clinical trials are necessary to establish clear indications for these potential uses.

2.2.1.2. Physico-chemical properties

Below, is a comprehensive compilation of the physico-chemical properties of artesunate found in the International Pharmacopoeia ([Ph. Int, 2022a](#)). To better visualise its structure, refer to Figure 2.2 accompanying this information.

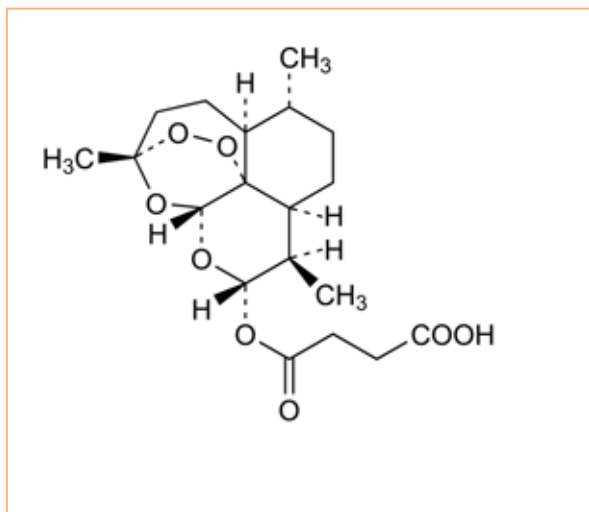


Figure 2.2: Chemical structure of artesunate ([Clarke's Analysis of Drugs and Poisons, 2021a](#)).

Molecular formula: C₁₉H₂₈O₈ ([Ph. Int, 2022a](#))

Molecular weight: 384.4 g/mol ([Ph. Int, 2022a](#))

Identification: Fine, white crystals or powder ([Ph. Int, 2022a](#)).

Melting point: 140 – 142 °C ([ACS, 2023](#))

pKa: 4.68 at 37 °C using potentiometric titration with 0.05 N sodium hydroxide ([Naik, 2007](#)).

Solubility: It is very slightly soluble in water, freely soluble in acetone and ethanol, and very soluble in dichloromethane ([Ph. Int, 2022a](#)).

BCS Classification: Class II ([Chadha et al., 2011](#)).

2.2.2. Pyrimethamine

Pyrimethamine is the most potent of all the 2–4–diaminopyrimidines. Since the 1940s, pyrimethamine has been tested for anti-malarial activity and has shown potent inhibitory activity. It is mostly used in combination therapy with other antimalarials, such as sulfadoxine, forming the popular sulfadoxine – pyrimethamine (SP) regimen ([Shapiro & Goldberg, 2006](#)).

2.2.2.1. Pharmacology

To enable the survival, growth, and development of the malaria parasite, the construction of DNA depends on the presence of folic acid, also known as vitamin B9. During the production of DNA (shown in Figure 2.3), p-aminobenzoic acid (PABA) is converted to dihydrofolic acid (DHF), a process catalysed by the enzyme, dihydropteroate synthase (DHPS). Subsequently, dihydrofolic acid is transformed to tetrahydrofolic acid (THF), the active form of folic acid, by an enzyme known as dihydrofolate reductase (DHFR). Tetrahydrofolic acid is vital to produce nucleotide building blocks, such as purines, pyrimidines and amino acids, for DNA ([Rosenthal, 2018](#); [Scholar, 2009](#); [Pubchem, 2023a](#)).

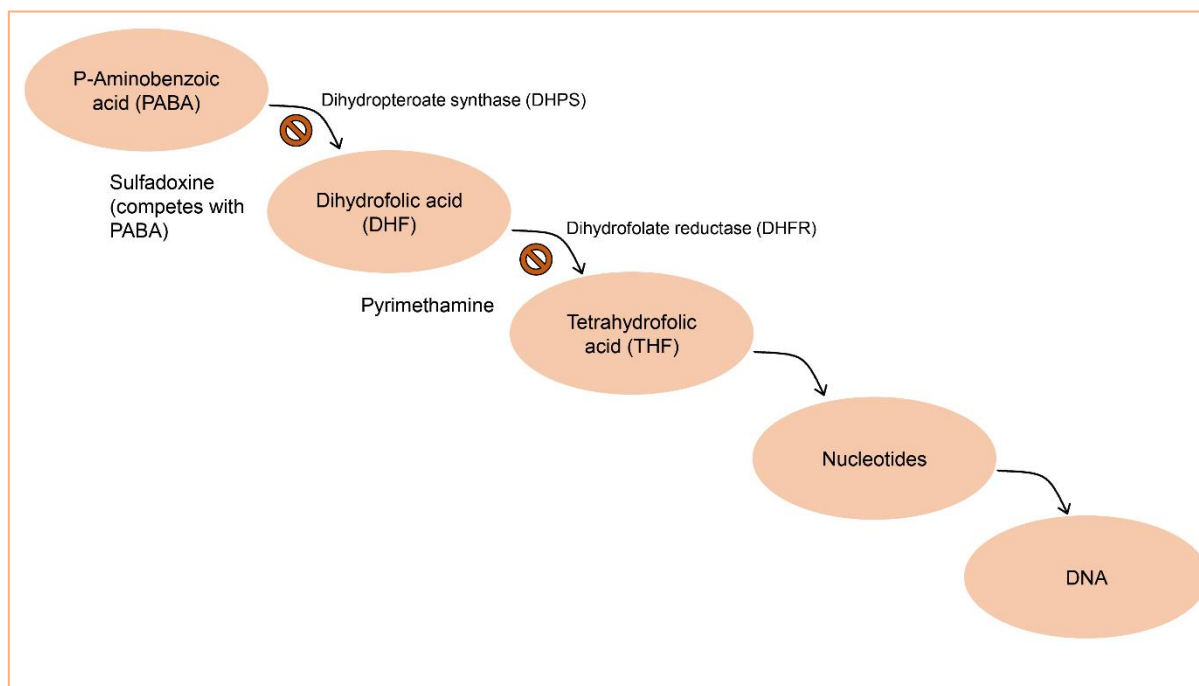


Figure 2.3: *De novo* pathway for folate synthesis in *P. falciparum* ([Rosenthal, 2018](#)).

Pyrimethamine acts by irreversibly binding to the active site of the enzyme DHFR. By doing so, it effectively prevents the conversion of DHF to THF. This disruption causes a deficiency in THF, which in due course impairs the parasites to produce sufficient nucleotides, hindering the replication of genetic material. Insufficient DNA leads to the death of the parasite or the prevention of its spread ([Rosenthal, 2018](#); [Scholar, 2009](#); [Pubchem, 2023a](#)).

Pyrimethamine is widely indicated to treat various parasitic diseases. Its primary indication includes seasonal prophylaxis against *Plasmodium falciparum* malaria and as the treatment for acute uncomplicated malaria. Pyrimethamine is commonly employed in combination with sulfonamides specifically. Combination therapy is encouraged by the WHO, due to the slow onset of acting and thus a greater chance of resistance. Further, it is crucial to manage toxoplasmosis, a disease caused by the parasite *Toxoplasma gondii* ([Rosenthal, 2018](#); [Scholar, 2009](#); [Pubchem, 2023a](#)). The combination pyrimethamine with sulfadiazine, is first-line treatment for toxoplasmosis, specifically in patients with suppressed immune systems, such as those who are HIV positive. Alternatively, in cases where patients have allergies to sulfonamides or to address toxicities associated with sulfadiazine, pyrimethamine is used in combination with clindamycin ([Shapiro & Goldberg, 2006](#)).

2.2.2.2. Physico-chemical properties

Below, the physico-chemical properties attributed to pyrimethamine is presented as found in the International Pharmacopoeia ([Ph. Int, 2022b](#)), along with Figure 2.4 displaying its molecular structure.

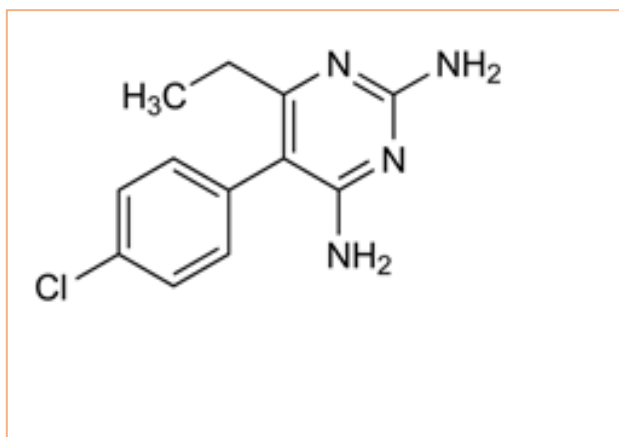


Figure 2.4: Chemical structure of pyrimethamine ([Clarke's Analysis of Drugs and Poisons, 2021b](#)).

Molecular formula: $C_{12}H_{13}ClN_4$ ([Ph. Int, 2022b](#))

Molecular weight: 248.71 g/mol ([Ph. Int, 2022b](#))

Identification: White, unscented, and tasteless crystalline powder ([Ph. Int, 2022b](#)).

Melting point: 233 °C – 243 °C ([Ph. Int, 2022b](#))

pKa: 7.34 at 20 °C ([Alaa Eldin Refat et al., 2022](#)).

Solubility: Pyrimethamine is practically insoluble in water ([BP, 2022a](#)). Furthermore, it is slightly soluble in ethanol, hydrochloric acid (HCl), acetone and chloroform ([Loutfy & Aboul-Enein, 1983](#)). In a study done by [Song et al., \(2022\)](#), it was found that pyrimethamine is much more soluble in alcohols with straight chains than in those with branched chains.

BCS Classification: Class II or IV according to the BCS ([Duque et al., 2017](#)).

2.2.3. Sulfadoxine

Sulfonamides were discovered in the early 1900s and was a turning point in the treatment of bacteria. Sulfadoxine was synthesised in the mid 1940s with the hope of fighting off bacterial infections. Later on, remarkable anti-malarial properties were discovered and explored. In the 1960s combination therapy was introduced to enhance the efficacy of sulfadoxine. Combination therapy included pyrimethamine ([Shambaugh, 1966](#); [Laing, 1964](#); [Laing 1965](#)).

2.2.3.1. Pharmacology

Sulfadoxine is an ultra-long-acting sulfonamide with broad-spectrum activity. It exhibits structural similarity to PABA, which enhances its effectiveness in treating microorganisms capable of synthesising their own folate. Sulfadoxine compete with PABA by binding to the active site of the enzyme DHPS, thus inhibiting the production of dihydrofolic acid, an important intermediate in the *de novo* pathway (refer to Figure 2.3 in section 2.2.2.1 described under pyrimethamine). This disruption hinders the formation of purines, pyrimidines and amino acids, leading to the blockade of DNA and RNA production. As a result, cell growth is halted and the parasite perishes ([Pubchem, 2022b](#); [Ferone, 1977](#); [Irvine, 2020](#); [Beauduy & Winston, 2018](#)).

2.2.3.2. Physico-chemical properties

Provided below are the physico-chemical properties of sulfadoxine as per the monograph in the International Pharmacopoeia ([Ph. Int, 2022g](#)), accompanied by Figure 2.5, illustrating its molecular structure.

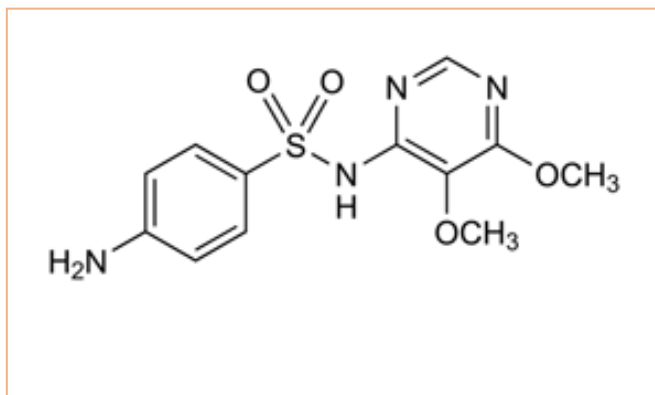


Figure 2.5: Chemical structure of sulfadoxine ([Clarke's Analysis of Drugs and Poisons, 2021c](#)).

Molecular formula: C₁₂H₁₄N₄O₄S ([Ph. Int, 2022g](#))

Molecular weight: 310.33 g/mol ([Ph. Int, 2022g](#))

Identification: Odourless crystalline powder that is yellowish-white or white ([Ph. Int, 2022g](#)).

Melting point: Approximately 198 °C with decomposition ([BP, 2022b](#)).

pKa: Hekster & Vree performed a study on the pharmacokinetics of sulphonamides, and reported a pKa value of 6.3 for sulfadoxine in physiological pH ([Hekster & Vree, 1982](#)).

Solubility: It is very slightly soluble in water, and slightly soluble in methanol and ethanol. Practically insoluble in ether ([BP, 2022b](#); [Ph. Int, 2022g](#)). Additionally, it is soluble in dilute mineral acids and solutions of alkali hydroxides and carbonates ([Kapoor, 1988](#)).

BCS Classification: Class II or IV ([van Wyk, 2022](#)).

2.3. Biopharmaceutical Classification System (BCS)

Solubility is one of the most important aspects of APIs with regard to drug-development. To comprehend the predominant importance of an API's absorption in the matter of solubility and permeability under specified circumstances, a theoretical system was developed by [Amidon et al. \(1995\)](#) known as the Biopharmaceutical Classification System (BCS). This system provides a correlation between theoretical *in vitro* testing and *in vivo* bioavailability studies ([Amidon et al., 1995](#)).

Initially, the objective was to aid in the regulatory oversight of post-approval alterations and generics, based solely on *in vitro* data, where applicable. The BCS is currently used during the early development of APIs to predict dissolution, solubility, and permeability to enhance pharmacokinetic parameters by implementing various oral API delivery techniques ([Kunde et al., 2015](#); [Sachan et al., 2009](#)). The key parameters, aqueous solubility and gastrointestinal permeability, classify APIs into four categories as shown in Table 2.2 below.

Table 2.2: Categories of the BCS and their characteristics ([Amidon et al., 1995](#))

BCS Category	Characteristics
I	High solubility; high permeability
II	Low solubility; high permeability
III	High solubility; low permeability
IV	Low solubility; low permeability

Class I: This class of APIs are highly soluble and highly permeable. When the maximum single therapeutic dose of an API dissolves in 250 mL or less of aqueous media over a 1.2 to 6.8 pH range at a temperature of 37 °C with minimal variation, it is considered to be highly soluble ([WHO, 2017](#)). Dissolution is usually the rate limiting step in API absorption. However, the dissolution profile must be well-defined because if an API is rapidly dissolving, the gastric emptying rate is the rate limiting step. In fasted state the gastric emptying is dependent on the volume and the motility phase ([Amidon et al., 1995](#); [Kunde et al., 2015](#)). For a multisource product to be classified as very rapidly dissolving, a minimum of 85 % of the indicated API

amount must dissolve within 15 min at 37 ± 1 °C, using a paddle or basket apparatus in up to 900 mL of specific media at pH 1.2, pH 4.5, and pH 6.8, without the inclusion of surfactants but with the potential use of enzymes in the presence of gelatine. Similarly, to be labelled as rapidly dissolving, a multisource product should see at least 85 % of the stated API quantity dissolve within 30 min under the same conditions and considerations as mentioned for very rapidly dissolving products ([WHO, 2017](#)). Furthermore, the WHO, classify an API as highly permeable if its absorption in humans is 85 % or higher ([WHO, 2017](#)).

Class II: This class APIs have a low solubility and high permeability. Dissolution rate is the rate limiting step, except when an API is given at a very high dose. The dissolution rate is usually enhanced, by mostly formulating slow-release APIs or use of enhancement techniques ([Amidon et al., 1995](#); [Kunde et al., 2015](#)).

Class III: APIs within this class dissolve very rapidly; resulting in a higher solubility but low permeability. The permeability has a significant impact on the APIs' capacity to be absorbed, thus permeation is the rate limiting step. The rate can consist of a high variation due to the change of the gastro-intestinal tract (GIT) physiology and membrane permeability. This group of APIs requires permeability improvement ([Amidon et al., 1995](#); [Kunde et al., 2015](#)).

Class IV: This class APIs are rarely developed and marketed due to the poor solubility-permeability profile and highly variable bioavailability. When these APIs are developed, drug delivery technologies are mostly used to enhance the bioavailability ([Amidon et al., 1995](#); [Kunde et al., 2015](#)).

The purpose of the BCS is to distinguish APIs which are eligible for bio waiving *in vivo* bioequivalence studies and to apply *in vitro* dissolution tests as a substitute for *in vivo* bioequivalence studies. Biowaiver means to relinquish expensive and time-consuming bioavailability and bioequivalence studies ([Kunde et al., 2015](#)). In order for APIs to be considered for possible waiving, the WHO created a “biowaiver list” with APIs classified as BCS Class I and III, as well as selected weakly acidic Class II APIs. The WHO showed interest to prequalify pyrimethamine, a BCS class II or IV API, for bio waiving ([WHO, 2020a](#); [Hofsäss & Dressman, 2019](#)).

2.4. Active pharmaceutical ingredient solubility

According to [Price and Patel \(2021\)](#), the term bioavailability refers to the degree (rate and fraction) to which an API becomes entirely accessible to its proposed biological area(s) of action, whether it is the site of action or the bodily fluid. Bioavailability can be influenced by physico-chemical and dosage form factors. These physico-chemical factors include pKa, dissolution rate, chemical stability, lipid solubility, and complexation potential ([Ashford, 2018a](#)).

Drug solubility is a characteristic of which an API forms a physically and chemically homogeneous mixture with other substances, such as solid APIs, in a solvent. Solubility is the concentration (when equilibrium is reached) of the solute in a saturated solution at any given temperature. Thus, it is the maximum amount (mass or volume) of solute that will dissolve in a given or fixed amount of solvent to produce a solution at a certain temperature and equilibrium ([Sachan et al., 2009](#); [Aulton, 2018](#)). Table 2.3 provides the various descriptions of API solubility.

Table 2.3: Descriptive terms used in solubility ([Aulton, 2018](#))

Descriptive term	Parts solvent needed for 1 part solute
Very soluble	Less than 1
Freely soluble	1 to 10
Soluble	10 to 30
Sparingly soluble	30 to 100
Slightly soluble	100 to 1000
Very slightly soluble	1000 to 10 000
Practically insoluble or insoluble	More than 10 000

There are different factors that can influence the solubility of an API. These factors are discussed below and could be employed as guidelines to improve the solubility of Class II and IV APIs ([Aulton, 2018](#)).

2.4.1. Temperature and heat input

When an API is administered orally, the terms dissolution and solubility work in tandem. The dissolution process is generally endothermic, which means heat is absorbed. When additional heat is applied, a positive change in enthalpy is seen, and more energy is available to break the intermolecular forces in the solute, making it easier for the solute to dissolve. This improves the APIs' solubility and is an example of Le Chatelier's principle if the system is in equilibrium. The converse, or an exothermic dissolution reaction, also holds true, but to a lesser extent. Heat will be released during an exothermic dissolution process. When additional heat is applied, the extra energy can oppose the process, leading to a decrease in solubility ([Aulton, 2018](#); [Le Chatelier, 1884](#)).

2.4.2. Molecular structure of the solute

The intermolecular interactions between the solute molecules and the solvent molecules play a vital role in determining whether a substance will dissolve and to what extent. Changes in this molecular structure of compounds or APIs, can have substantial impact on the solubility. When certain molecules such as hydrophilic hydroxyl groups or a salt of a weak acidic API are introduced, it can lead to an increase of solubility due to increased interactions between the solute and solvent ([Aulton, 2018](#)). An example where salt formation is used to improve solubility can be described with the API quinine. Quinine sulphate is slightly soluble in water ([Ph. Int., 2022f](#)). Quinine hydrochloride is soluble in water ([Ph. Int., 2022e](#)). Quinine bisulphate is freely soluble in water and quinine dihydrochloride is very soluble in water ([Ph. Int., 2022c](#); [Ph. Int., 2022d](#)).

However, in certain cases, APIs are esterified, resulting in decreased solubility but offering other advantageous properties. This includes, masking the taste of the API, protecting the API from degradation in the GIT or to enhance the absorption of the API ([Aulton, 2018](#)).

2.4.3. Nature of solvent

A conjecture of the term “good” solvent is typically based on the “like dissolves like” principle. This means that a solute will dissolve better in a solvent with comparable chemical properties, meaning polar solutes dissolves better in polar solvents, and non-polar solutes dissolves better in non-polar solvents. Water is most commonly used in pharmaceutical formulations, due to lack of toxicity and low costs ([Aulton, 2018](#); [Murdan, 2018](#)).

The effect of co-solvents was investigated in a study by Nayak & Panigrahi (2012), with the aim to improve the aqueous solubility of etoricoxib. The co-solvents used during this study included polyethylene glycol (PEG) 400, glycerin and polypropylene glycol (PG). Among the solvent-cosolvent combinations tested, water-PEG 400 presented with the highest solubility enhancement potential. Co-solvents are two or more solvents mixed together to create a solvent system with unique properties. Co-solvents can impact solubility by altering the polarity or solvation properties of the primary solvent. They may also help solubilise a solute that is only sparingly soluble in the main solvent. Co-solvents are essential tools in pharmaceutical formulation to improve the solubility and stability of an API, and to enhance drug delivery options, but it must be acceptable and non-toxic to humans ([Murdan, 2018](#)).

2.4.4. Particle size

It is assumed that the solubility of an API is influenced by its particle size according to [Aulton \(2018\)](#). This effect can be attributed to the electrical charge on the particles. When the particle size is reduced, there is a greater surface area of contact between the solute and solvent. This can facilitate a faster dissolution rate, and an increase in equilibrium solubility. Many poorly soluble, slow dissolving APIs are micronised to increase the surface area. Consequently, this raises the saturation point, allowing more solute to be accommodated by the solvent.

However, with some hydrophobic APIs, such as phenobarbital, a reduction in particle size cause agglomeration. Agglomeration is when the particles are too small and forms clusters. As a result, the surface area decreases, and it is difficult for the solvent to reach the solute. The solubility and bioavailability will decrease. Fortunately, this can be addressed by using wet-milling techniques or employing nanotechnology to reduce the particles to nano size ([Ashford, 2018a](#); [Khadka et al., 2014](#)).

2.4.5. pH

The different pH levels in the gastro-intestinal tract can significantly affect solubility-pH dependent drugs. The pH of the oesophageal lumen varies between 5 and 6 and decreases to a pH variation of 1 to 3.5 in the stomach. An increase in the luminal pH of the small intestine varies from 6 to 7.5. The colon pH levels range from 6 to 7.5, specifically in the caecum 6 to 6.5 and an increase between 7 and 7.5 in the distal parts of the colon ([Ashford, 2018b](#)). The pKa value of the API, the pH of the fluid in the specific location, and the permeability coefficient of the API all influence the ratio of unionised to ionised API molecules in solution in the

gastrointestinal system. The degree of ionised API molecules influence solubility and thus the absorption capability. The pH of the gastrointestinal tract in which the molecule is present and its pKa value affect how much it ionises ([Aulton, 2018](#)).

One useful approach to estimate solubility and permeability is with the help of Lipinski's rule of 5. An APIs' poor solubility is influenced by a number of factors, including the drug partition coefficient (Log P greater than 5), chemical structure i.e, more than 5 hydrogen bond donors and the sum of -N and -O groups, also known as hydrogen bond acceptors (> 10), the molecular weight (> 500), the likelihood that the compound will serve as a substrate for P-glycoprotein efflux ([Lipinski et al., 2012](#)).

In a study performed by [Sieger et al. \(2017\)](#), the pH-dependent solubility and permeability profiles were investigated of different APIs, as this is also a useful tool to establish oral bioavailability. If the sole factors affecting bioavailability are solubility and permeability, pH-dependent solubility and permeability profiles provide a practical way to forecast bioavailability following oral administration. The parameters for the best oral bioavailability can be determined from a pH-window that is created by combining both pH-dependent solubility and pH-dependent permeability in one figure. Twenty-one compounds, each with a known oral bioavailability, were used to establish the correlation between the size of the pH-window and the observed oral bioavailability. As examples, nintedanib (a dibasic API) showed enhanced solubility in an acidic media and low solubility in a neutral media. Meloxicam (an acidic API) depicted poor solubility in an acidic media and enhanced solubility in a neutral media. In conclusion, solubility and permeability are displaying contrasting patterns for ionisable molecules. In the pH range where permeability is excellent, the unionised, often weakly soluble form is the dominating species, whereas in the pH range where solubility is good, charged species with low permeability rule the solubility behaviour. It was then found that compounds with a pH-window < 2 displayed poor oral bioavailability (< 25 %). It was also established that if the pH-window increased with 1 pH-unit, the oral bioavailability increased within the region of 25 % ([Sieger et al., 2017](#)). This correlates to the pH-partition theory. The absorption of an API will depend on the extent of its unionised form at the site of absorption. According to the pH-partition theory, a weakly basic drug is more likely to be absorbed from the intestine (higher pH), and a weakly acidic drug from the stomach, a lower pH range ([Ashford, 2018a](#)).

2.4.6. Solid–state properties

Crystallisation is the process by which molecules come together in a specific arrangement to form a solid crystal structure ([Boistelle & Astier, 1988](#)). It can occur under different conditions which result in different polymorphic forms, or the formation of hydrates and solvates. Polymorphism is when various crystalline forms of the same substance occur, each with their own lattice energies and other important properties such as solubility and stability ([Buckton, 2018](#); [Byrn et al., 1999](#); [Saifee et al., 2009](#)).

A polymorph that is the most thermodynamically stable is often the least soluble. In some formulations the metastable solid-state forms are preferred especially if it is medically beneficial. These benefits can include, faster dissolution rate, and the ability to achieve higher concentrations for rapid absorption. Metastable forms are often more soluble, making them suitable for pharmaceutical formulation to achieve acceptable systemic exposure for APIs with low solubility. However, it is important to note that metastable forms gradually convert back to the more stable polymorphic form during the API's shelf life ([Buckton, 2018](#), [Saifee et al., 2009](#)). Amorphism is the absence of a crystalline structure. Amorphous forms lack a melting point as they do not possess a crystal lattice to break and leads to an improved solubility compared to that of a crystalline structure ([Buckton, 2018](#)).

2.5. Compatibility of active pharmaceutical ingredients

Compatibility is a critical point in the pre-formulation, manufacturing, and administration of APIs, more so during combination therapy in malaria disease due to the resistance of the parasite to monotherapy. Additionally, having multiple APIs to administer orally for the treatment of a disease can lead to decreased patient adherence ([Jeličić et al., 2021](#)). When APIs are combined with each other and/or excipients, it is important to ensure product stability, bioavailability, and safety by assessing the interactions of the components with each other ([Veiga et al., 2017](#)). All APIs have different properties, such as solubility, pH, crystalline structure, dissolution rate, and bioavailability. When APIs are combined, these factors can become either complementary or conflicting interactions. Incompatibility is considered when interactions (meant to improve chemical or physical modification) conflict and form towards a less desirable state ([Patel et al., 2015](#)). This could possibly lead to an API becoming dormant or inactive, resulting in a decrease in therapeutic efficacy and safety ([Dourado, 2019](#)).

Various tests are available to evaluate the physical, chemical, and therapeutic compatibility of APIs used in combination. Chemical compatibility tests include Fourier Transform Infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), Thermal activity microcalorimetry (TAM), and High-performance liquid chromatography (HPLC). DSC measures the thermal behaviour of components, such as changes in peaks and variations in enthalpy, during analysis to identify interactions that may result in melting point shifts or heat reactions. It is assumed that components are compatible if the sum of the individual components correlates to the thermal properties of the mixture. However, DSC results alone can be misleading; thus, additional analysis is needed to understand the interactions between the APIs ([Patel et al., 2015](#)). FT-IR is a method used in complementary conjunction with thermal analysis to detect changes in chemical bonds and identify potential chemical reactions between different APIs and/or excipients ([Dinte et al., 2013](#)). HPLC is employed to monitor the degradation of APIs and the formation of impurities in the presence of excipients. However, stress testing through HPLC to determine incompatibility can be time-consuming and thermal analysis is rather encouraged ([Patel et al., 2015](#)). It is important to ensure artesunate, pyrimethamine and sulfadoxine are compatible, since the WHO encourages these three APIs to be used in a fixed-dose combination (FDC) ([WHO, 2021](#)).

2.6. Summary

Malaria has posed a significant challenge, despite various endeavors aimed at preventing, treating, or eradicating the disease. The success of these interventions is hindered by the rapid evolution of the malaria parasite, leading to the development of resistance. With the escalating concern of resistance, combination therapies have been introduced as a strategic response ([Ingraham & Ingraham, 2004](#); [WHO, 2022](#)). This led researchers to establish the compatibility of APIs before an FDC formulation. Compatibility ensures that the efficacy outweighs the risks and that patients adhere ([Wilkins et al, 2024](#)) during the treatment of malaria.

The solubility of APIs is crucial for bioavailability and is influenced by various factors, such as the structure-, size-, and solid-state properties of the solute (API) and, the pH and temperature of the environment of the API ([Aulton, 2018](#)). These factors offer guidelines and insights for enhancing the solubility of certain class APIs, such as artesunate, pyrimethamine, and sulfadoxine, all of whom are classified as Class II or IV (see Section 2.2. Active pharmaceutical ingredients, subsections 2.2.1.2; 2.2.2.2; 2.2.3.2 respectively). Furthermore, the interaction that two or more APIs might have on each other's solubility profiles may provide the necessary

information required for the development of a new FDC as is recommended by the WHO in this case ([WHO, 2021](#)).

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CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

In the context of pharmaceutical development, the solubility of APIs holds immense significance as it provides a foundation for the formulation of effective drug products. Determining the solubility profiles are crucial to guaranteeing the desired levels of therapeutic efficacy, safety, and bioavailability when the API is administered as a therapeutic treatment.

In addition to the effective development and formulation of APIs, compatibility is also crucial when administered orally. Compatibility studies are usually performed between APIs and their excipients and, to a lesser extent, between different APIs indicated for the same disease or administered in an FDC. It is important to recognise that the APIs can potentially influence one another's physico-chemical properties, such as stability, dissolution rate, and solubility ([Patel et al., 2015](#)).

Pyrimethamine and sulfadoxine, while having a longstanding presence in the market, are facing the mounting challenge of resistance. To address the issue of resistance, the WHO integrated and supported the use of artesunate with pyrimethamine and sulfadoxine in the "list of essential medicines" ([WHO, 2021](#)). The solubility of artesunate, pyrimethamine, and sulfadoxine were investigated (separately and in combination) in various media over a pH range of 1.2, 4.5, and 6.8 to establish if any of these APIs were influenced by the presence of another.

Hereafter, physico-chemical characterisation and compatibility studies were completed to provide insight and possible explanations regarding the API's behaviour. Physico-chemical studies are used to identify interactions, either between APIs or APIs and their excipients, or to explore an API's structural and thermal aspect. These tests were performed using Differential Scanning Calorimetry (DSC), Fourier Transform Infrared spectroscopy (FT-IR), and Thermal Activity Monitoring (TAM). In this chapter, the methods used during this study to perform all the tests mentioned above, are discussed.

3.2. Materials

All the materials (chemicals and APIs) used for this study were of analytical or high-performance liquid chromatography (HPLC) grade and procured from reliable South African wholesalers. For filtration, 0.45 µm pore size filters were employed. Table 3.1 represents the APIs used during this study. Several in-house water purification systems produced ultrapure water with a resistivity of at least 18.1 megaohm.

Table 3.1: List of APIs used in this study

Material	Manufacturer
Artesunate	DB Fine Chemicals (Pty) Ltd. (Johannesburg, South Africa)
Pyrimethamine	DB Fine Chemicals (Pty) Ltd. (Johannesburg, South Africa)
Sulfadoxine	DB Fine Chemicals (Pty) Ltd. (Johannesburg, South Africa)

3.3. Research Design

During this study, the APIs were prepared for the tests, which are described in sections 3.4 – 3.5. Table 3.2 lists the various APIs and their combinations as well as the tests performed on each. Throughout the investigation, these combinations were employed to examine the impact the various APIs had on the solubility behaviour of each other. All tests included the three separate compounds (single components) as a standard of comparison against the combination of the various APIs. Please note that for the purpose of this study, the main focus was on pyrimethamine and the influence both sulfadoxine and artesunate had individually on this API and vice versa, since it has been established in a study performed by [Badenhorst \(2017\)](#) that a possible intermolecular interaction occurs between sulfadoxine and pyrimethamine. Furthermore, the ratios investigated during solubility testing were those of the comb-pack kit, while for the physico-chemical characterisation a 1:1 ratio was used, as this proved to be the best ratio to test for incompatibilities. A fair study of potential drug-drug incompatibility is made easier when the APIs are prepared in a 1:1 ratio ([Botha & Letter, 1990](#)).

Table 3.2: The study design

Combination of the APIs	Studies done
Artesunate (A)	Solubility, DSC, FT-IR, TAM
Pyrimethamine (P)	Solubility, DSC, FT-IR, TAM
Sulfadoxine (S)	Solubility, DSC, FT-IR, TAM
AP	Solubility, DSC, FT-IR, TAM
SP	Solubility, DSC, FT-IR, TAM

***FDC:** Fixed dose combination as per WHO, 2021, **DSC:** Differential scanning calorimetry, **FT-IR:** Fourier transform infrared, **TAM:** Thermal activity monitoring

3.4. Methods

3.4.1. High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a key analytical technique in the field of pharmaceutical sciences, renowned for its exceptional precision and versatility. It is a robust method for separating, identifying, and quantifying compounds within complex mixtures ([Hitachi, 2023a](#)). Two HPLC systems were employed to investigate the solubility profiles of artesunate, pyrimethamine, and sulfadoxine. Artesunate is inherently unstable in solution; thus, the methodological strategy was to analyse artesunate from a different HPLC-system than that of pyrimethamine during the solubility testing for the artesunate-pyrimethamine combination in order to analyse both of the APIs simultaneously.

3.4.1.1. HPLC method validation

In literature, validation is defined as the process of proving an analytical method is adequate and consistent for the intended purpose. To confirm that the method aligns with the specified requirements, validation entails a series of laboratory studies and documented evidence. In essence, it is the action of verifying that a given method, equipment, material or system consistently produce the expected results, hence guaranteeing that particular procedures will result in output that meets predetermined standards ([Huber, 1998](#)). Validation is based on 8

key parameters (described in Table 3.3 below), to assess the performance characteristics of a method.

Table 3.3: Key parameters of method validation ([Huber, 1998](#); [ICH, 2022](#))

Parameter	Description
Accuracy	The degree of proximity between the results obtained from an analytical method and the true reference values (Huber, 1998). According to the ICH guideline (ICH, 2022), a method is considered accurate when the percentage relative standard deviation (%RSD) ≤ 2 , the system had proven linearity ($R^2 \geq 0.99$).
Precision	The ability to constantly yield highly reproducible and dependable results. Three separate components make up accuracy: reproducibility, intermediate precision, and repeatability (Huber, 1998). Repeatability was done by carrying out six to nine determinations for three matrices at different concentrations in a short period of time to evaluate the method's precision. Intermediate precision extends this examination over a period of time, examining the performance in a single laboratory under a variety of settings (ICH, 2022).
Specificity/selectivity	Selectivity and specificity are used interchangeably (Huber, 1999). A method is deemed specific if it accurately detects and quantifies the target analyte amidst potential interfering substances. Selectivity, on the other hand, refers to the method's capacity to generate responses of a number of chemical entities, whether discernible from one another or not. However, if it is distinguished between the entities, the method is considered selective (Huber, 1998 ; ICH, 2022). In HPLC, selectivity was obtained through optimal columns, precise chromatographic conditions, such as mobile phase composition, column temperature, and detector wavelength. It

	was also obtained with sample preparation: Blank injections of all the solvents were analysed respectively to ensure that there was no simultaneous elution with the APIs. Furthermore, cross-injections of the APIs onto each other's methods were performed to establish the peak elution positions.
Linearity and range	It ensures that the correlation among the concentration of analytical substances in samples and the related test outcomes are directly proportional within a predetermined range (Huber, 1998). This was measured by generating a calibration curve and analysing standards of varied concentrations. The correlation coefficient (R^2) was calculated to assess the linearity. A calibration curve is considered linear if the $R^2 \geq 0.99$. The range for validation in this study can be expressed either as concentration or as percentage range. The percentage range was calculated for each API in each of the solvents. The criteria for range require at least 80 % to 120 % (ICH, 2022).
Limit of detection (LOD)	The LOD represents the lowest concentration that can be detected, although not necessarily enumerated. In HPLC, it can be determined by the peak with a height at least two or three times as high as the baseline-noise level. Besides the signal-to-noise level (S/N) method, there are three more ways to determine the LOD. It can be calculated through visual inspection; by analysing blank samples and calculating the standard deviation of the responses to determine the amount of analytical background; or alternatively, through the linearity equation based of the calibration curve (Huber, 1998; ICH, 2022). In this study, the latter method was employed. LOD can be expressed as: $\text{LOD} = (3.3 \times \sigma)/S$ Where: σ: Standard deviation of the response (y-intercept) S: Slope of the calibration curve

Limit of quantitation (LOQ)	LOQ represents the lowest analyte concentration that can be quantified with acceptable accuracy and precision (Huber, 1998). From the calibration curve, LOQ was determined using few variables in the linearity equation and can be expressed as: $\text{LOQ} = (10 \times \sigma)/S$ Where: σ: Standard deviation of the response (y-intercept) S: Slope of the calibration curve
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3.4.1.2. HPLC – sulfadoxine and pyrimethamine

A Hitachi Chromaster (Tokyo, Japan) chromatographic system, which comprised a 5410 UV detector, an auto-sampler (5260) with a sample temperature controller, and a solvent delivery module (5160) was used in this study (Figure 3.1). To examine data, the HPLC Chromaster system manager was utilised ([Hitachi, 2023c](#)). The column used for analysis was a Phenomenex Luna C18 (250 mm x 4.6 mm). Sulfadoxine and pyrimethamine were both analysed using the same analytical method and mobile phase.



Figure 3.1: HPLC system used to analyse sulfadoxine and pyrimethamine ([Hitachi, 2023b](#)).

Mobile phase. Acetic acid glacial (20 ml) was dissolved in 1.6 L of ultra-purified (UP) water. To this solution, 1 ml of triethylamine was added. The pH was adjusted with a 10 M sodium hydroxide solution to pH 4.2 and sufficient UP-water was added to total a final volume of 20 L. Then, 400 ml of acetonitrile was added to 1.6 L of this solution. The final solution was degassed and filtered ([Ph. Int, 2022e](#)).

Method set-up. The HPLC, was then programmed and conditioned with a C18 column (chemically bonded octadecyl silyl groups – 3 μm) at 25°C. It operated at a flow rate of 1.5 ml per min. The detector was set at a wavelength of 210 nm – 254 nm. Every injection volume was 20 μl and a runtime of 25 min was employed – this method was adapted from the International Pharmacopoeia ([Ph. Int, 2022e](#)).

3.4.1.3. HPLC – artesunate

VWR Hitachi Chromaster HPLC system was used to analyse the samples acquired artesunate during this study (displayed in Figure 3.2). This system (5160) comprised of a photodiode array detector (5430), an auto-sampler (5260) with a sample temperature controller (5310), and a solvent supply. To examine data, Data Apex, Clarity VA software was utilised. The column used to analyse artesunate was Phenomenex Luna C18 (250 mm x 4.6 mm).



Figure 3.2: Adapted version of the HPLC system used to analyse artesunate ([Hitachi, 2023c](#)).

Mobile phase. A ratio of 44 volumes of acetonitrile and 56 volumes of buffer at pH 3.0 was used for artesunate. A buffer with a pH of 3.0 was produced by dissolving 1.36 g of potassium

dihydrogen phosphate with 900 ml of water. Then, phosphoric acid (85% w/w) was added to the mixture to adjust the pH to 3.0. Finally, the solution was adjusted to a final volume of 1000 ml with water ([Ph.Int, 2022b](#)).

Method set-up. The HPLC, was then programmed and conditioned with a C18 column (chemically bonded octadecyl silyl groups – 3 μm) at 30°C. It operated at a flow rate of 1 ml per min. The detector was set at a wavelength of 216 nm. Every injection volume was 20 μl and a runtime of 15 min was set ([Ph. Int, 2022b](#)).

3.4.2. Solubility studies

For solubility testing, a water bath containing an aluminium rotating axis (54 rpm) and a temperature regulator was used (as displayed in Figure 3.3). The temperature was set to 37°C ($\pm 2^\circ\text{C}$). During the preliminary studies, samples of a predetermined weight were individually weighed in test tubes for artesunate, sulfadoxine, and pyrimethamine, and 10 ml of selected buffer/solution was pipetted into each tube to form super-saturated solutions. This was done in triplicate for each API (individually and in combinations) at each sampling time (2, 4, 8, 12, 24 and 48 h). To avoid leaking, Parafilm[®] was used to seal and secure the test tubes, and a screw cap was firmly attached to each test tube. Hereafter, each test tube was attached to the rotating axis in the water bath and set to rotate for 48 h. During each sampling, the rotating axis motor was switched off, and the samples were left to settle. Samples were taken from each test tube at every time interval, filtered (0.45 μm), and analysed using HPLC. The process was repeated at pH 1.2, 4.5, and 6.8, and solubility investigations were conducted using various buffer/solutions as specified in section 3.3.2. The purpose of the preliminary studies was to establish the trend of each API's solubility profile and to guarantee that equilibrium was attained during the 48 h period, furthermore, it may give insights on possible instability.

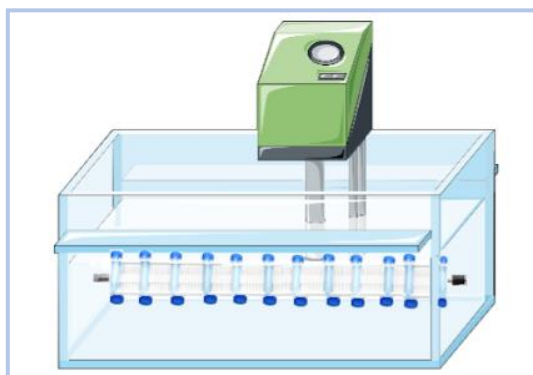


Figure 3.3: Adapted version of solubility water bath ([Smart, 2023; Hurst, 2023](#)).

Following the preliminary studies, the pivot solubility experiment (6 extractions at pH 1.2, 4.5, and 6.8) commenced, with the parameters remaining constant but the duration of the solubility study decreasing to an appropriate period after equilibrium. This was done to guarantee that the APIs' exposure time to various media/buffers were as short as possible, to ensure optimum results after equilibrium with the least amount of degradation possible.

3.4.2.1. Artesunate

A supersaturated solution for artesunate was created by weighing approximately 50 mg of the drug into individual test tubes and adding it with 5 ml of buffer or solution for both the preliminary and pivot solubility investigations. At every time interval, three samples were withdrawn, filtered, and HPLC-analysed.

3.4.2.2. Pyrimethamine

Approximately 50 mg of pyrimethamine was weighed into separate test tubes for both the preliminary and pivot solubility investigations, and 5 ml of buffer or solutions was pipetted into each tube to create a supersaturated solution for the drug. At every time interval, three samples were withdrawn, filtered, diluted, and followed by HPLC analysis.

3.4.2.3 Sulfadoxine

To create a supersaturated solution for sulfadoxine, individual test tubes containing 50 mg of sulfadoxine were weighed and then filled with 5 ml of buffer or solution for both the preliminary and pivot solubility investigations. At every time interval, three samples were withdrawn, filtered, and HPLC – analysed.

3.4.2.3. Solubility of APIs in combination

To test the influence of the APIs on each other, solubility studies were also performed in combination. It was decided to combine the APIs as it would be used commercially, according to the [WHO \(2021\)](#).

- 50 mg Artesunate, 25 mg pyrimethamine
- 25 mg Pyrimethamine, 500 mg sulfadoxine

The above-mentioned combinations were weighed in test tubes and then filled with 5 ml of buffer or solution for both preliminary and pivot studies. At every time interval, three samples were withdrawn, filtered and HPLC – analysed.

3.4.3. Buffers and solution preparation

The preparation of the buffers and solutions (pH 1.2 – 6.8) used in this study were done so in accordance with that of the British Pharmacopoeia (BP) and International Pharmacopoeia (Ph. Int.). These buffers and solutions were prepared as described in sections 3.4.3.1 to 3.4.3.6.

3.4.3.1. 0.1 N Hydrochloric acid solution (HCl), pH 1.2

A 0.1 N solution was prepared by diluting 10.08 ml of hydrochloric acid (32 % v/v) to 1000 ml with carbon dioxide-free water ([Ph. Int., 2022a](#); [BP, 2022](#)).

3.4.3.2. Sodium chloride buffer solution (NaCl), pH 1.2

2 g sodium chloride was dissolved in 800 ml water. Hydrochloric acid (70 g/l) was used to decrease the pH of the solution to 1.2. The solution was then diluted to 1000 ml with water ([Ph. Int., 2022a](#); [BP, 2022](#)).

3.4.3.3. Acetate buffer solution (Ace), pH 4.5

2.99 g of sodium acetate was dissolved in 900 ml of water, and the pH adjusted to 4.5 by adding approximately 14 ml of acetic acid (120 g/l). Finally, the solution was diluted to 1000 ml using water ([Ph. Int, 2022a](#); [BP, 2022](#)).

3.4.3.4. Potassium phosphate buffer solution (PBS), pH 6.8

To produce a 0.2 M solution, 27.22 g of potassium dihydrogen phosphate (monobasic potassium phosphate) was dissolved in 1000 ml of water. 50 ml of this solution was then transferred to a 200 ml volumetric flask. Sodium hydroxide solution (10 N) was added to bring the pH to 6.8. Thereafter, the solution was diluted to a final volume of 200 ml with water ([Ph. Int, 2022a](#); [BP, 2022](#)).

3.4.3.5. Sodium phosphate buffer solution (SBS), pH 6.8

6.9 g of sodium dihydrogen phosphate and 0.9 g sodium hydroxide were dissolved in 800 ml of water. The pH was then adjusted to 6.8 with sodium hydroxide solution (10 N) and diluted with water to 1000 ml ([Ph. Int, 2022a](#); [BP, 2022](#)).

3.4.3.6. Water

Ultra-purified water was accessible in-house.

3.4.4. Statistical analysis

3.4.4.1. Statistical analysis of preliminary studies

Analysis of covariance (ANCOVA) was used to determine the difference between the two solubility profiles, of each single component and its relevant combination, on a statistically significant level. For this study, the assumption of parallel regression lines was foregone, and the following model (Eq. 1) was selected:

$$y_{ij} = \alpha_i + \beta_i x_{ij} + \epsilon_{ij} ; j = 1, \dots, n_i ; i = 1, 2 ; \quad \text{Eq. 1}$$

where ϵ_{ij} are independent $n(0; \sigma^2)$ variates.

In this model, we estimated (α_1, β_1) and (α_2, β_2) independently (Eq.'s 2 - 3), but σ^2 jointly as Eq. 4:

$$\hat{\beta}_i = \frac{\sum_j y_{ij}(x_{ij} - \bar{x}_i)}{\sum_j (x_{ij} - \bar{x}_i)^2} \quad \text{Eq. 2}$$

$$\hat{\alpha}_i = \bar{y}_i - \hat{\beta}_i \bar{x}_i. \quad \text{Eq. 3}$$

$$\hat{\sigma}^2 = \frac{\sum_i \sum_j (y_{ij} - \hat{\alpha}_i - \hat{\beta}_i x_{ij})^2}{(n_1 + n_2 - 4)} \quad \text{Eq. 4}$$

The responses at time zero and at time n_i , were tested pairwise for the solubility data to see if they were equal. Therefore, the hypothesis tested for the solubility data was $H_0: \alpha_1 + \beta_1 x = \alpha_2 + \beta_2 x$, compared to the test statistic:

$$f_x = (\hat{\alpha}_1 + \hat{\beta}_1 x - \hat{\alpha}_2 - \hat{\beta}_2 x)^2 / \hat{\sigma}^2 \left[\frac{1}{n_1} + \frac{(x - \bar{x}_1)^2}{\sum (x_{1j} - \bar{x}_1)^2} + \frac{1}{n_2} + \frac{(x - \bar{x}_2)^2}{\sum (x_{2j} - \bar{x}_2)^2} \right] \quad Eq. 5$$

with $F_{\alpha;1;n_1+n_2-4}$. Testing at $\alpha = 0.05$, any values of $f_x > F_{crit}$ will therefore be indicative of a statistically significant difference.

3.4.4.2. Statistical analysis of pivot studies

To investigate the influence of the different API combinations and buffer solutions on the aqueous solubility of the APIs used in this study, a non-parametric two-way analysis of variance (ANOVA) in the form of an aligned ranks transformation ANOVA (ARTANOVA) was performed ([Wobbrock et al., 2011](#); [Elkin et al., 2021](#)). Effect sizes were calculated using the partial eta-squared (η^2) metric and interpreted as proposed by [Cohen \(1988\)](#). *Post hoc* tests were conducted using pairwise Mann-Whitney U tests with Bonferroni adjustments of the p-values. ARTANOVA was performed using the ARTool package ([Wobbrock et al., 2011](#)) in R (version 4.1.3) as seen in [R Core Team \(2022\)](#). The ARTANOVA model fit results suggested an excellent fit, with an Efron R^2 of 0.998, a mean absolute percent error (MAPE) of 0.0415% and coefficient of variation (CV) of 5.81%.

3.4.5. Physico-chemical characterisation

The following instrumental approaches were employed to investigate the physico-chemical characteristics of artesunate, sulfadoxine, and pyrimethamine alone and in various combinations (see section 4.2.3):

- Differential Scanning Calorimetry (DSC)
- Fourier Transform Infrared (FT-IR)
- Thermal Activity Monitoring (TAM)

3.4.5.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is used to gain information about the melting point and any energy changes that may be associated with phase transformations. The DSC thermograms were generated by employing a Mettler DSC 3+ instrument (Mettler Toledo, Greifensee, Switzerland) as displayed in Figure 3.4. Samples weighing approximately 3 to 5 mg were positioned in aluminium crimp cells (40 μ l) with punctured lids and heated to the required

temperature (max 300°C) at a heating rate of 10°C/min; with a nitrogen gas purge rate of 35 ml/min.



Figure 3.4: Differential scanning calorimetry ([DSC, 2023](#)).

3.4.5.2. Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy was used to attain information on the potential intermolecular interactions and behaviour of the solid-state coupled APIs. This approach is an effective method recommended for identifying pure compounds and the compatibility between APIs ([Segall, 2019](#)). As shown in Figure 3.5, the Shimadzu IR Tracer-100 spectrophotometer (Shimadzu, Kyoto, Japan) was utilised to capture infrared spectra. The samples were placed on a QATR 10 accessory and scanned 64 times over a range of 400 – 4000 cm^{-1} . The data was then processed using LabSolutions version 1.108.



Figure 3.5: Fourier transform infrared spectroscopy ([Shimadzu, 2023](#)).

3.4.5.3. Thermal activity microcalorimetry

During this study, a 2277 Thermal Activity Monitor (TAM) (TA Instruments, USA) was used to investigate the interaction between artesunate, sulfadoxine, and pyrimethamine and to link the effects observed during solubility determinations with heat flow data acquired by means of microcalorimetry. Compatibility was established between APIs by using a 2277 Thermal Activity Monitor (TAM III) (Thermometric AB, Sweden) with an oil bath with a stability of 100 K over 24 h (displayed in Figure 3.6). All samples were packed in ampoules and sealed with rubber stoppers and aluminium crimp closures. The microcalorimetry was carried out at 40°C. The heat flow was measured for single APIs as well as for the combinations. The instrument is sensitive to detecting potential incompatibilities and instabilities between APIs. Readings produced by the monitor that exceeds 2 to 3 joules indicate possible interactions, resulting in undesirable findings. Findings below 2 to 3 joules prove the absence of interactions.



Figure 3.6: Thermal Activity Monitor ([TAM, 2023](#)).

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CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

During the development of medications, it is imperative to formulate FDCs containing APIs and excipients that are compatible with one another. The importance hereof lies in the quality, safety, and efficacy of FDCs from preformulation to manufacturing and ultimately administration ([Veiga et al., 2017](#)). To ensure this, three anti-malarial APIs (artesunate, pyrimethamine, and sulfadoxine) were investigated regarding their solubility profiles and physico-chemical characterisation (as single components and in combination) to establish compatibility for FDC formulation. In this chapter, all results obtained from method validation, to solubility studies, and finally physico-chemical characterisation are discussed and scrutinised.

4.2 HPLC method validation

During HPLC method validation for this study, the following parameters from the [ICH-guidelines \(2023\)](#) were employed: accuracy, precision, specificity and selectivity, linearity, limit of detection (LOD), and limit of quantitation (LOQ). Each of these parameters have a criterion for acceptance as described in Chapter 3 subsection 3.4.1.1.

4.2.1 Artesunate method validation in different buffer solutions

Precision: A minimum of 5 to 7 standards featuring different concentrations were prepared and subjected to 3 to 6 injections per standard. This was done for solvents: acetate (ACE), potassium phosphate (PBS), sodium phosphate (SBS), and water. Precision was established through the attainment of repeatability, with all the relative standard deviations (% RSD) having been found to be below 2 %. However, for 0.1 N HCl solution and the NaCl buffer solution, only one injection per standard was used to establish the calibration curve due to the instability of artesunate ([Al Haydar, 2011](#); [Bezuidenhout, et al., 2022](#)). For the same reason, intermediate precision could not be established.

Specificity: The method for artesunate analysis demonstrated specificity and selectivity through several measures. Blank injections, including 0.1 N HCl solution, NaCl, ACE, PBS, and SBS buffers, and water showed no peaks at the 10 to 11 min retention time when artesunate

was eluted. Pyrimethamine standards as cross-over injections on the artesunate HPLC-method in the above-mentioned buffer solutions revealed no interference. The pyrimethamine's retention time was between 2 and 3 min, depending on the type of buffer solution (refer to Annexure A).

Linearity and range: The calibration curves for artesunate in various solvents and buffers are displayed in Figure 4.1. It was apparent from the R^2 values obtained that all regression coefficients complied with the criteria (R^2 -value ≥ 0.99). For HCl solution and NaCl buffer solution, the range was 80 $\mu\text{g/ml}$ – 1200 $\mu\text{g/ml}$. For buffer solutions ACE, PBS, and SBS, the range was between 200 $\mu\text{g/ml}$ – 1200 $\mu\text{g/ml}$. Lastly, the range for water was 200 $\mu\text{g/ml}$ – 2000 $\mu\text{g/ml}$.

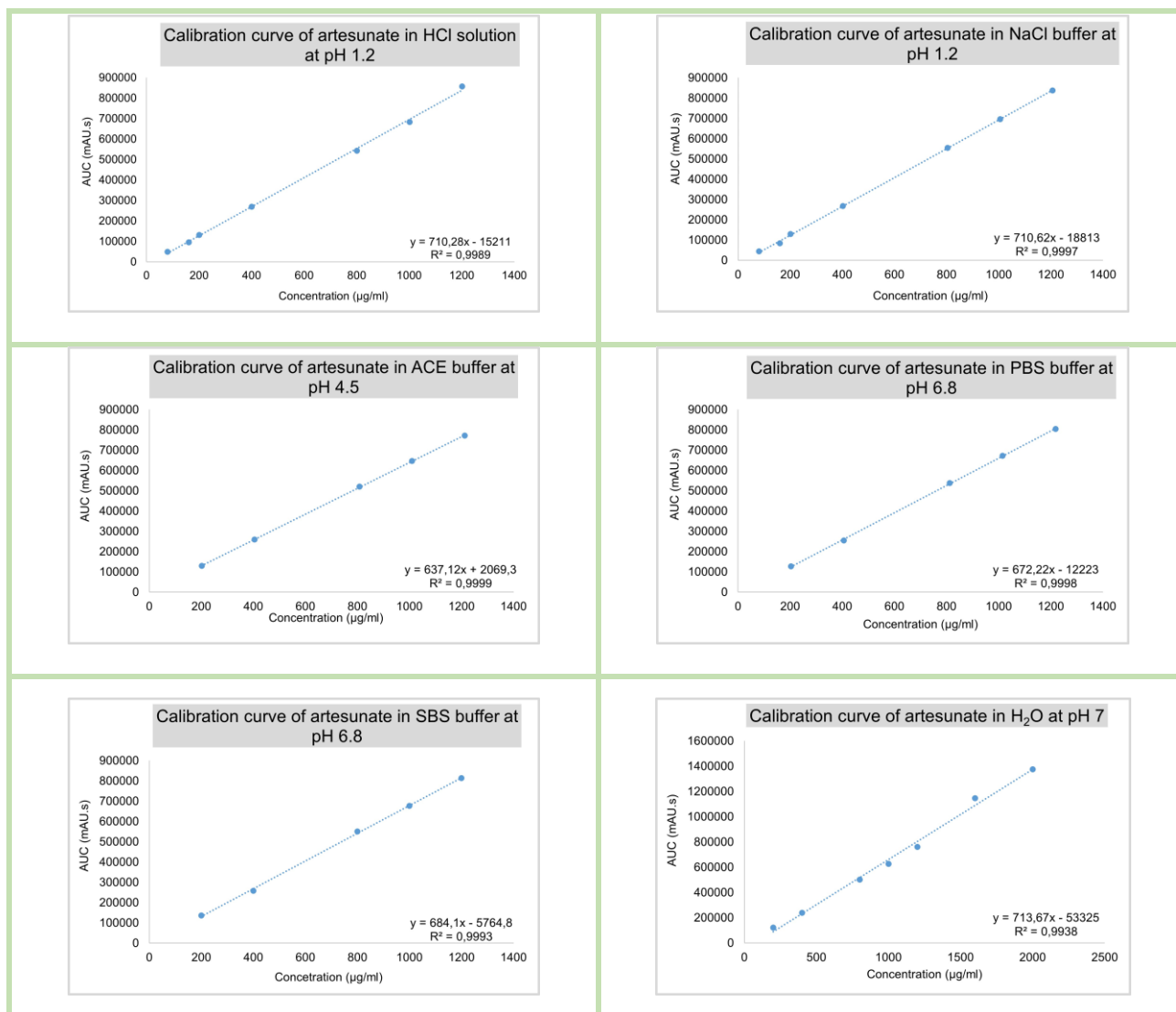


Figure 4.1: Calibration curves of artesunate in various solutions and buffers.

Limit of detection: It was calculated using the linearity equation. The detection limit of the artesunate method was the following in each solvent: 0.1 N HCl solution 34.24 µg/ml, NaCl 18.30 µg/ml, ACE 14.58 µg/ml, PBS 24.11 µg/ml, SBS 39.63 µg/ml, and water 137.96 µg/ml.

Limit of quantitation: It was calculated using the linearity equation. The quantitation limit of the artesunate method was the following in each solvent: 0.1 N HCl solution 103.76 µg/ml, NaCl 55.46 µg/ml, ACE 44.18 µg/ml, PBS 73.07 µg/ml, SBS 120.09 µg/ml, and water 418.07 µg/ml.

Accuracy: Accuracy may be inferred due to the establishment of the precision, linearity, and specificity of the method in all media/solvents.

4.2.2 Pyrimethamine method validation in different buffer solutions

Precision: Five standards featuring different concentrations were prepared and subjected to 3 – 6 injections. This was done for all the solvents. Precision was established through attainment of repeatability, with the relative standard deviations (% RSD) having been found to be below 2 %. However, at the lowest concentration (2.56 µg/ml) for all solvents, the %RSD was slightly higher than 2 % due to this concentration being close to the quantitation limit. Intermediate precision was achieved with the preliminary and pivot solubility experiments, by making use of the middle concentration obtained from the calibration curve as a standard. Precision was achieved, by confirming that the area under the curves (AUC) for the concentration standards used during the solubility experiments correlated to the same concentrations' AUC obtained in the calibration curves. This was consequently done for all solutions, buffer solutions, and water during the pyrimethamine single component, S + P combination, and A + P combination.

Specificity: Blank injections, including 0.1 N HCl solution, NaCl, ACE, PBS, SBS buffers, and water showed no peaks at the 18 to 21 min retention time when pyrimethamine was eluted (refer to Annexure B). Artesunate standards were injected as cross-over on the pyrimethamine method in the above-mentioned buffers. It is clear (in Annexure B) that although artesunate and pyrimethamine elute close to one another in certain buffers/solutions, there was no noteworthy interference.

Linearity and range: The calibration curves, as seen below in Figure 4.2, for pyrimethamine in each solvent show that the R^2 -values were above 0.99 and acceptable according to the criteria. For all solvents (HCl, NaCl, ACE, PBS, SBS, and water), the concentration range was between 2.56 $\mu\text{g/ml}$ and 38.4 $\mu\text{g/ml}$.

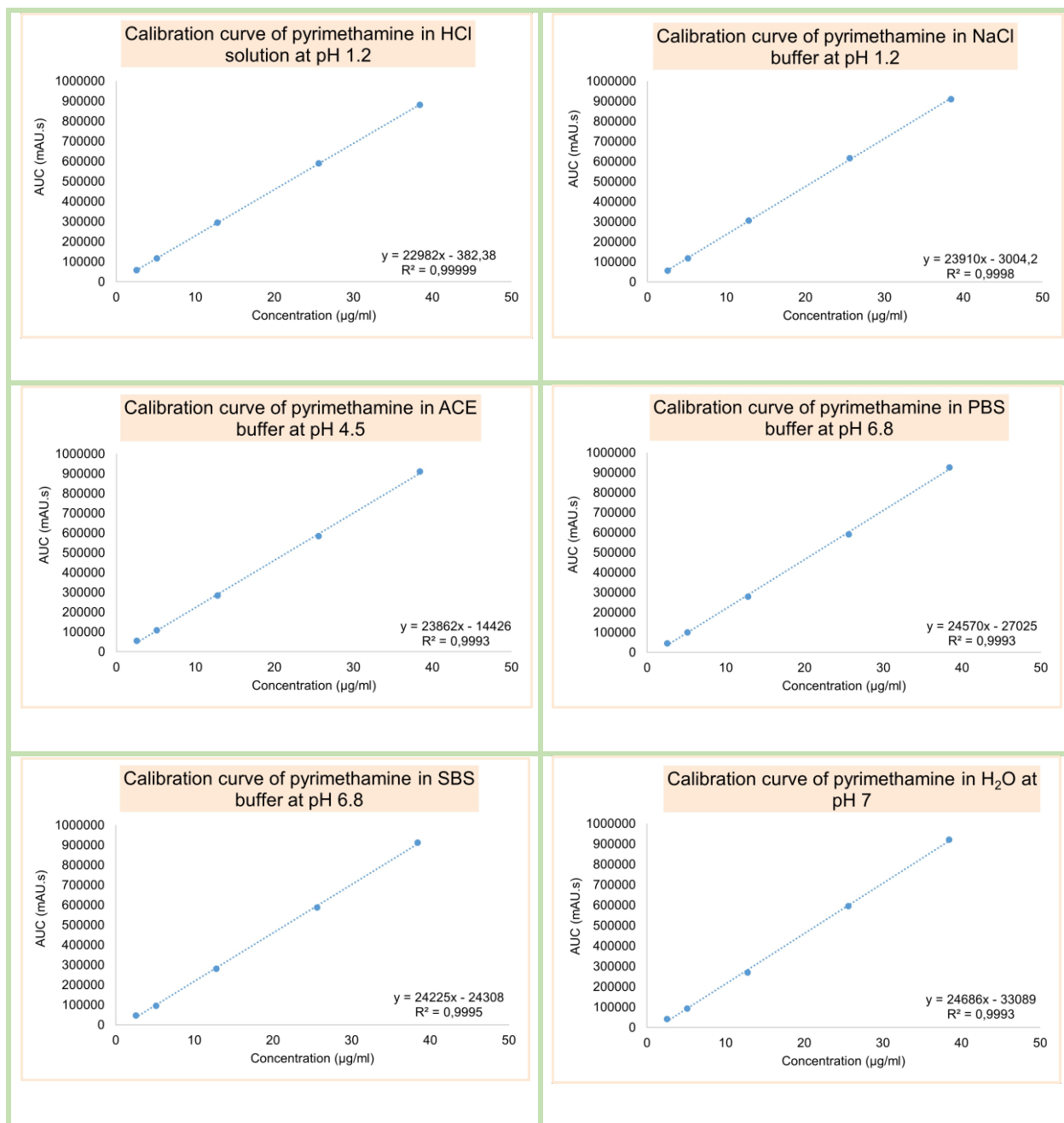


Figure 4.2: Calibration curves of pyrimethamine in various solutions and buffers.

Limit of detection: It was calculated using the linearity equation. The detection limit of the pyrimethamine method was the following in each solvent: 0.1 N HCl solution 0.10 µg/ml, NaCl 0.53 µg/ml, ACE 1.05 µg/ml, PBS 1.18 µg/ml, SBS 0.90 µg/ml, and water 1.05 µg/ml.

Limit of quantitation: It was calculated using the linearity equation. The detection limit of the pyrimethamine method was the following in each solvent: 0.1 N HCl solution 0.30 µg/ml, NaCl 1.60 µg/ml, ACE 3.18 µg/ml, PBS 3.59 µg/ml, SBS 2.74 µg/ml, and water 3.18 µg/ml.

Accuracy: Since linearity, precision, and specificity were established, the system was deemed accurate in all the media and solvents.

4.2.3 Sulfadoxine method validation in different buffer solutions

Precision: Between 5 and 7 concentrations were prepared and subjected to 3 – 6 injections. This was done for all the solvents. Precision was established through the attainment of repeatability, with the relative standard deviations (% RSD) having been found to be $\leq 2\%$. However, with lower concentrations (4 µg/ml and 20 µg/ml) in the HCl solution, NaCl-, and ACE-buffer solutions, and water, the %RSD exceeded 2% due to variations in the peak area, likely attributed to the proximity to LOD or LOQ. Using the middle concentration from the calibration curve as a benchmark, intermediate accuracy was achieved in the preliminary and pivot solubility experiments. By verifying that the concentration standards' AUCs during the solubility experiments correlated with the concentrations' AUCs established in the calibration curve, precision was attained. This was carried out for the sulfadoxine single component and, S + P combination in all solutions, buffer solutions, and water.

Specificity: The analytical method employed for pyrimethamine was also used to analyse sulfadoxine, establishing one specificity test (see pyrimethamine, subsection 4.2.2.). Sulfadoxine exhibited an elution at 12 min, with no peaks observed for all the blank injections (see Annexure B).

Linearity and range: The calibration curves, as seen below in Figure 4.3, for sulfadoxine in each solvent attained R^2 -values ≥ 0.99 according to the criteria. The concentration range in water, HCl solution, NaCl, and ACE buffer solutions were 4 µg/ml – 750 µg/ml. For PBS, and SBS the concentrations were between 50 µg/ml – 750 µg/ml.

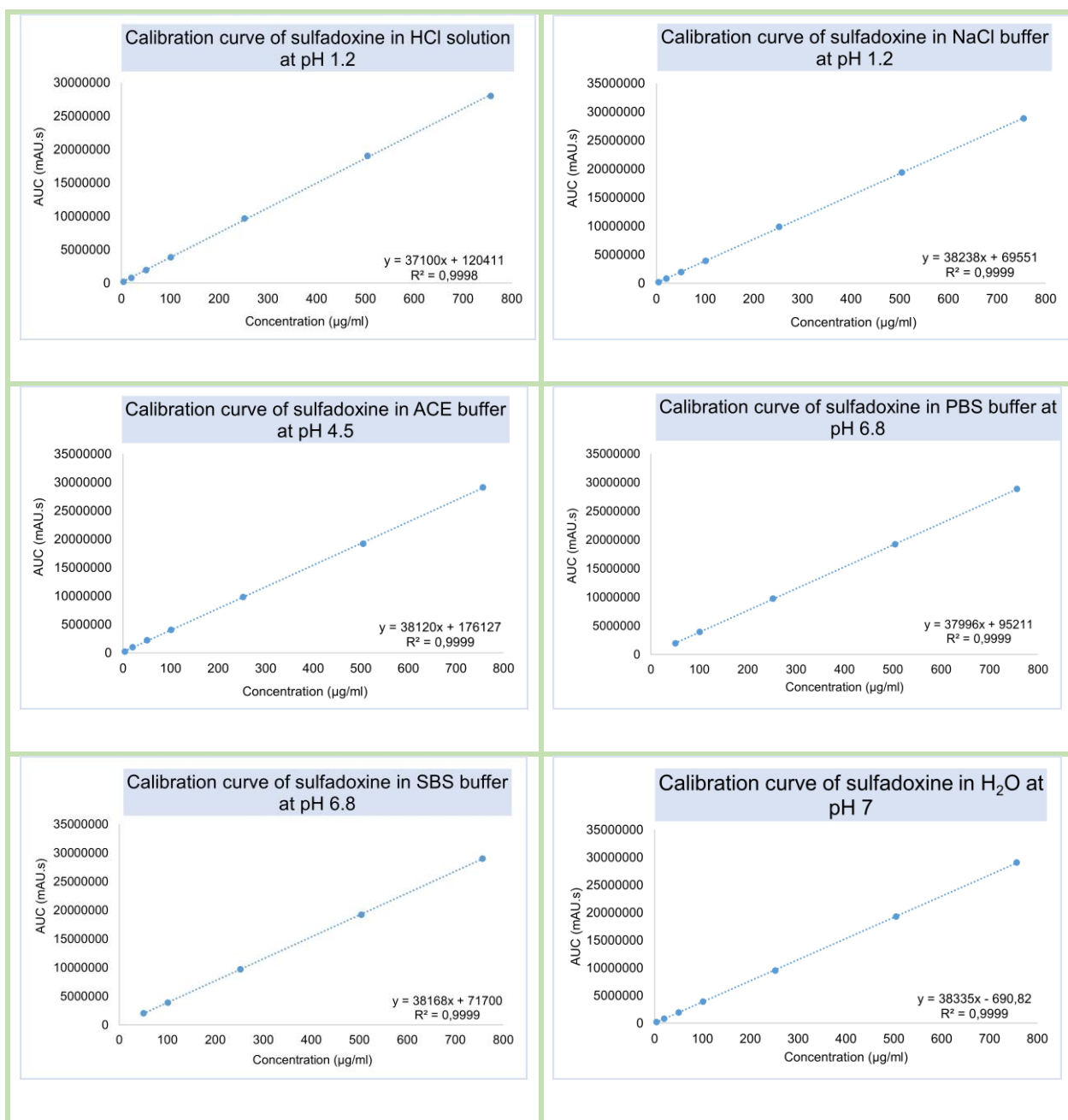


Figure 4.3: Calibration curves of sulfadoxine in various solutions and buffers.

Limit of detection: It was calculated using the linearity equation. The detection limit of the artesunate method was the following in each solvent: 0.1 N HCl solution 7.64 µg/ml, NaCl 3.87 µg/ml, ACE 5.77 µg/ml, PBS 3.99 µg/ml, SBS 4.79 µg/ml, and water 3.96 µg/ml.

Limit of quantitation: It was calculated using the linearity equation. The quantitation limit of the artesunate method was the following in each solvent: 0.1 N HCl solution 23.16 µg/ml, NaCl 11.72 µg/ml, ACE 17.5 µg/ml, PBS 12.09 µg./ml, SBS 14.51 µg/ml, and water 12.0 µg/ml.

Accuracy: As a result of the method's precision, linearity, and specificity being established in all media over the pH range 1.2 – 6.8, accuracy can be assumed.

4.3 Solubility studies

Solubility studies for each single component API (artesunate, pyrimethamine, and sulfadoxine) and for the combinations (Artesunate-pyrimethamine and pyrimethamine-sulfadoxine) were performed using the methodology described in Chapter 3 (see Subsection 3.4.2).

4.3.1 Artesunate

In this section artesunate as single component and artesunate in the combination with pyrimethamine (50 mg A + 25 mg P), are discussed. Preliminary solubility studies were performed in water and the various buffers/solutions over the pH range of 1.2 – 6.8. In all preliminary experiments, the pH of the solvents was measured before initiating the experiment. Then, the pH of the solution (solute + solvent) was taken afterwards at the various time intervals.

4.3.1.1 Preliminary solubility in 0.1 N HCl solution at pH 1.2

Preliminary solubility results obtained for artesunate (single component) in 0.1 N HCl solution are presented in Table 4.1 and Figure 4.4.

Table 4.1: Preliminary solubility data of artesunate in 0.1 N HCl at pH 1.2

Preliminary - Artesunate (Single) in HCl								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	AH1	212587	196023	204305	309,06	273,84	33,78	12,33
	AH2	182423	171759	177091	270,74			
	AH3	160489	152462	156476	241,72			
4 h	AH4	178542	168640	173591	265,81	235,44	28,50	12,11
	AH5	153910	144170	149040	231,25			
	AH6	138071	128786	133429	209,27			
8 h	AH7	131391	124413	127902	201,49	177,91	22,17	12,46
	AH8	113725	104112	108919	174,76			
	AH9	100188	93118	96653	157,49			
12 h	AH10	127337	123045	125191	197,67	177,74	20,83	11,72
	AH11	115587	108884	112236	179,43			
	AH12	98397	92952	95675	156,12			
24 h	AH13	0	0	0	0	0	0	0
	AH14	0	0	0	0			
	AH15	0	0	0	0			
48 h	AH16	0	0	0	0	0	0	0
	AH17	0	0	0	0			
	AH18	0	0	0	0			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **A:** Artesunate, **H:** HCl solution, **1 – 18:** Sample number.

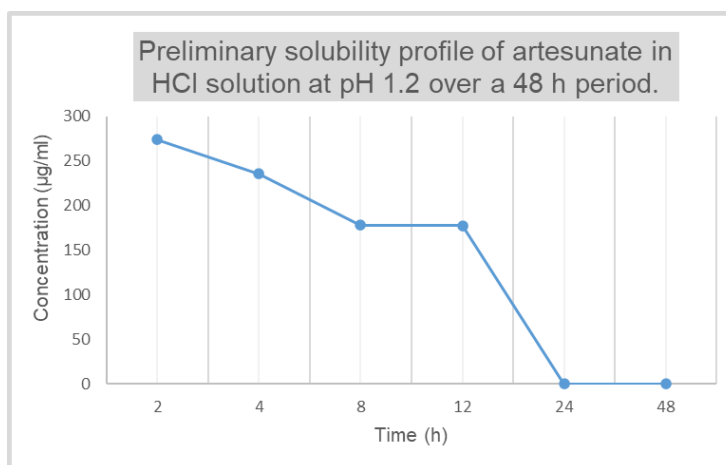


Figure 4.4: Preliminary solubility profile of artesunate (single component) in 0.1 N HCl solution.

It is clear that the artesunate single component obtained a maximum solubility of approximately 273 µg/ml at 2 h after which degradation of the API was apparent. At the 24 and 48 h intervals, the analysis of these samples proved below LOD as there were no artesunate observed. The % RSD for the 2 – 12 h time intervals were above 10%, possibly due to the rapid degradation of artesunate in a low pH environment. Similar results were found by [Bezuidenhout, \(2016\)](#), where the decrease in API concentration was attributed to rapid degradation in lower pH environment.

The preliminary solubility results obtained for artesunate (in A + P combination) in 0.1 N HCl solution are presented in Table 4.2 and Figure 4.5.

Table 4.2: Preliminary solubility data of artesunate in the A+P combination in 0.1 N HCl at pH 1.2

Preliminary - Artesunate (A + P combination) in HCl								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APH1	207311	311400	259356	386,56	310,23	68,30	22,02
	APH2	194931	185531	190231	289,24			
	APH3	175223	156432	165828	254,88			
4 h	APH4	109428	138757	124093	196,12	167,26	31,91	19,08
	APH5	127048	113601	120325	190,82			
	APH6	91133	75039	83086	138,39			
8 h	APH7	102320	76231	89276	147,11	128,28	25,86	20,16
	APH8	96057	70899	83478	138,94			
	APH9	57034	52879	54957	98,79			
12 h	APH10	156790	102966	129878	204,27	174,04	27,55	15,83
	APH11	100044	83117	91581	150,35			
	APH12	96317	111201	103759	167,50			
24 h	APH13	0	0	0	0	0	0	0
	APH14	0	0	0	0			
	APH15	0	0	0	0			
48 h	APH16	0	0	0	0	0	0	0
	APH17	0	0	0	0			
	APH18	0	0	0	0			

***AUC:** Area under the curve, **AVG:** Average area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering: A:** Artesunate, **P:** Pyrimethamine, **H:** HCl solution, **1 – 18:** Sample number.

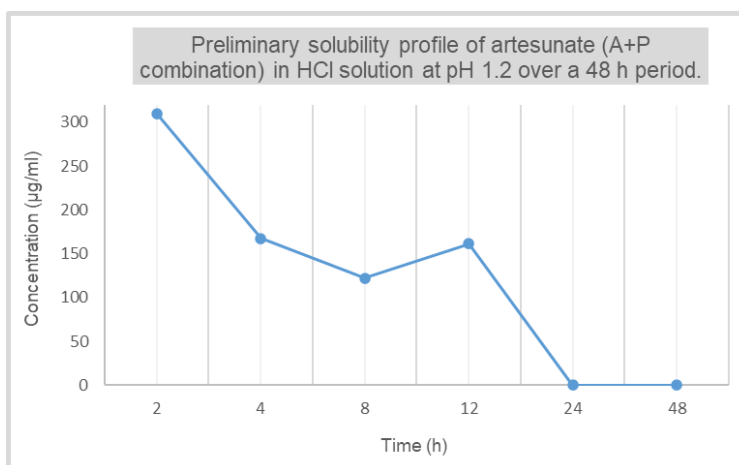


Figure 4.5: Preliminary solubility profile of artesunate (in A + P combination) in 0.1 N HCl solution.

The experimental data presented in Table 4.2 revealed key insights into the solubility and degradation behaviour of artesunate in combination with pyrimethamine in the 0.1 N HCl solution. In the A + P combination, artesunate reached a maximum solubility of ± 310 µg/ml within 2 h, after which degradation of the API occurred.

Furthermore, the degradation rate of artesunate in combination with pyrimethamine was observed to be higher between 2 – 4 h when compared to the single component. At the 24 and 48 h intervals, the analysis of these samples proved below LOD, as evident by the absence of artesunate peaks in the chromatograms.

The % RSD for the available data (2 – 12 h) exceeded 10% possibly due to the rapid degradation of artesunate in a low pH environment. At the 12 h interval, a spike in concentration was observed, which cannot be explained, and further investigation is needed in this regard. Please note that it was not the scope of this study to investigate these types of phenomena.

Regarding the pH profile of the single component in solution, a difference in the measurements were observed between pH 1.23 – 1.25 over the course of the experiment. In contrast, the pH profile of the A + P combination in solution was slightly higher, ranging from pH 1.30 – 1.40.

4.3.1.2 Preliminary solubility in NaCl buffer solution at pH 1.2

Preliminary solubility results obtained for artesunate (single component) in NaCl buffer solution are presented in Table 4.3 and Figure 4.6.

Table 4.3: Preliminary solubility data of artesunate in NaCl buffer solution at pH 1.2

Preliminary - Artesunate (Single) in NaCl								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	AN1	130812	122962	126887	205,03	182,41	21,98	12,05
	AN2	113154	106564	109859	181,07			
	AN3	98636	92743	95690	161,13			
4 h	AN4	108207	87392	97800	164,10	165,66	22,60	13,64
	AN5	104744	126246	115495	189,00			
	AN6	92167	74707	83437	143,89			
8 h	AN7	76483	74017	75250	132,37	117,78	14,14	12,01
	AN8	66724	61718	64221	116,85			
	AN9	56931	53434	55183	104,13			
12 h	AN10	81283	79578	80431	139,66	125,34	14,75	11,77
	AN11	73243	68425	70834	126,15			
	AN12	61748	57237	59493	110,19			
24 h	AN13	0	0	0	0	0	0	0
	AN14	0	0	0	0			
	AN15	0	0	0	0			
48 h	AN16	0	0	0	0	0	0	0
	AN17	0	0	0	0			
	AN18	0	0	0	0			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **N**: NaCl buffer solution, **1 – 18**: Sample number.

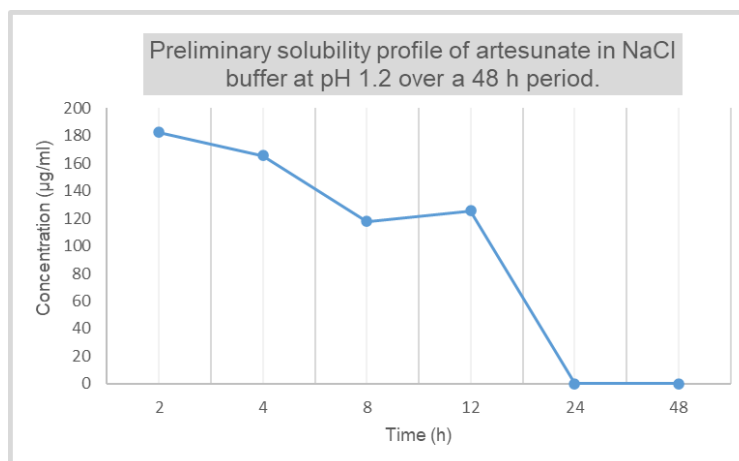


Figure 4.6: Preliminary solubility profile of artesunate (single component) in NaCl buffer solution.

It is evident that after 2 h, artesunate reached a maximum solubility of approximately 182 µg/ml, at which point the API began to degrade. The analysis of the 24 h and 48 h interval samples were below the LOD since there were no peaks in the chromatograms. The 2 – 12 h time intervals had a %RSD of more than 10% and could likely be attributed to the rapid degradation of artesunate in a low pH. In studies done by [Al Haydar \(2011\)](#) and [Bezuidenhout \(2016\)](#), respectively, both degradation and stability studies of artesunate in various media were performed, and it was also found that artesunate rapidly degrades in the lower pH ranges.

Artesunate, as the single component, measured a pH of 1.23 after the initial time interval of 2 h. Notably, the pH of 1.23 was maintained until 4 h whereafter variations were seen. However, it is important to note that with regards to the 0.1 N HCl solution, the pH wasn't maintained and varied from every time interval. It can also be noted that the artesunate degradation between time intervals in the 0.1 N HCl solution was more rapid than in the NaCl buffer solution. In the HCl solution, the difference in artesunate concentrations between 2 h and 4 h was about 38 µg/ml, whereas in NaCl it was slightly lower (16 µg/ml).

Preliminary solubility results obtained for artesunate (A + P combination) in NaCl buffer solution are presented in Table 4.4 and Figure 4.7.

Table 4.4: Preliminary solubility data of artesunate in the A+P combination in NaCl buffer solution at pH 1.2

Preliminary - Artesunate (A + P combination) in NaCl								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APN1	270072	222083	246078	372,76	347,57	22,77	6,55
	APN2	212912	234812	223862	341,50			
	APN3	203159	226031	214595	328,46			
4 h	APN4	45255	47041	46148	91,42	75,80	9,03	11,92
	APN5	37002	33895	35449	76,36			
	APN6	37135	32174	34655	75,24			
8 h	APN7	0	0	0	0	0	0	0
	APN8	0	0	0	0			
	APN9	0	0	0	0			
12 h	APN10	0	0	0	0	0	0	0
	APN11	0	0	0	0			
	APN12	0	0	0	0			
24 h	APN13	0	0	0	0	0	0	0
	APN14	0	0	0	0			
	APN15	0	0	0	0			
48 h	APN16	0	0	0	0	0	0	0
	APN17	0	0	0	0			
	APN18	0	0	0	0			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **N**: NaCl buffer solution, **1 – 18**: Sample number.

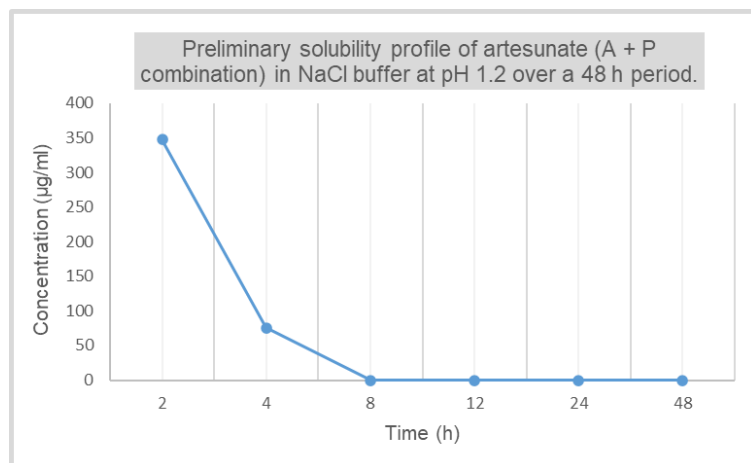


Figure 4.7: Preliminary solubility profile of artesunate (in A + P combination) in NaCl buffer solution.

The data presented in Table 4.4 revealed that artesunate (in A + P combination) in the NaCl buffer had a maximum solubility of 347 µg/ml within the initial 2 h, followed by a rapid escalation in degradation (even more so than for the 0.1 N HCl solution). Subsequently, during the analysis at 8, 12, 24, and 48 h time intervals, no peaks were eluting for artesunate, indicating complete degradation. The high %RSD is attributed to the degradation of artesunate during sample analysis.

In contrast to the single component, artesunate in A + P combination exhibited a higher initial concentration, with a difference of 165 µg/ml at 2 h between artesunate-single and artesunate in the A+P combination. Artesunate in combination with pyrimethamine displayed a more pronounced degradation rate than that of the single component.

Regarding the pH profiles, the single component demonstrated minimal variance, maintaining a pH range of 1.23 – 1.25 from the initial pH 1.2 throughout the 2 to 48 h period. In contrast, for the A + P combination, the pH profile fluctuated from a pH of 1.37 to a pH of 1.44. This discrepancy could be due to the weak basic properties of pyrimethamine (as described with the 0.1 N HCl solution in Section 4.3.1.2), leading to a gradual rise in pH.

4.3.1.3 Preliminary solubility in acetate (ACE) buffer solution at pH 4.5

Preliminary solubility results obtained for artesunate (single component) in ACE buffer solution are presented in Table 4.5 and Figure 4.8.

Table 4.5: Preliminary solubility data of artesunate in ACE buffer solution at pH 4.5

Preliminary - Artesunate (Single) in ACE								
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	AVG. Conc. (µg/ml)	SD	%RSD
2 h	AA1	347341	360393	353867	552,87	569,97	15,31	2,69
	AA2	375124	370290	372707	582,39			
	AA3	365968	369568	367768	574,65			
4 h	AA4	407121	412739	409930	640,72	630,35	11,59	1,84
	AA5	404299	405056	404678	632,49			
	AA6	391828	398821	395325	617,83			
8 h	AA7	496453	493443	494948	773,95	698,11	107,25	15,36
	AA8	400529	395787	398158	622,27			
	AA9	No data						
12 h	AA10	393608	444882	419245	655,32	636,53	34,30	5,39
	AA11	392707	448343	420525	657,33			
	AA12	380824	383157	381991	596,94			
24 h	AA13	299616	296804	298210	465,65	464,31	5,07	1,09
	AA14	302376	297785	300081	468,58			
	AA15	295458	292096	293777	458,70			
48 h	AA16	358811	357097	357954	559,27	548,93	10,27	1,87
	AA17	353856	348675	351266	548,79			
	AA18	346011	343690	344851	538,74			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering: A:** Artesunate, **A:** ACE buffer solution, **1 – 18:** Sample number.

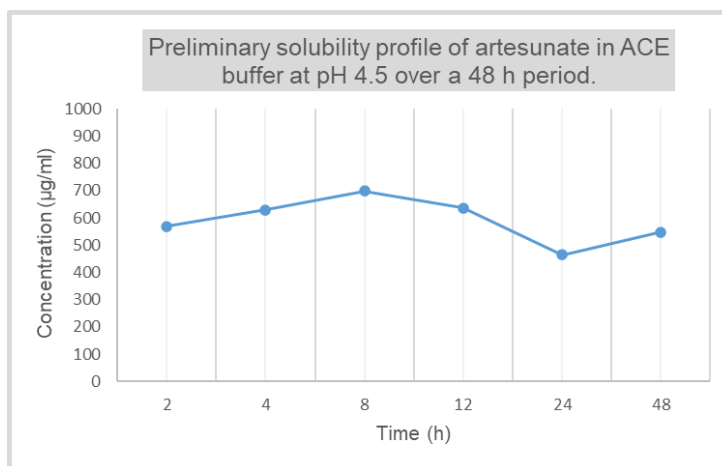


Figure 4.8: Preliminary solubility profile of artesunate (single component) in ACE buffer solution.

It is clear that artesunate in the single component obtained a maximum solubility of approximately 698 µg/ml at 8 h after which a slight decrease of the API was apparent in the ACE buffer. At the 48 h interval, the analysis of the samples resulted in a higher concentration, but this could be due to a possible degradation product co-eluting with the artesunate and not solely attributed to artesunate. The % RSD for the 8 h time interval was above 10%, due to a possible leak in one of the samples; hereafter, the adjusted average was calculated for a more accurate result.

Preliminary solubility results obtained for artesunate (A + P combination) in ACE buffer solution are presented in Table 4.6 and Figure 4.9.

Table 4.6: Preliminary solubility data of artesunate in the A+P combination in ACE buffer solution at pH 4.5

Preliminary - Artesunate (A + P combination) in ACE								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APA1	736186	684329	710258	1111,36	1063,46	43,98	4,14
	APA2	735985	611512	673749	1054,14			
	APA3	682944	627225	655085	1024,90			
4 h	APA4	708001	648231	678116	1060,99	1075,01	25,31	2,35
	APA5	738475	672944	705710	1104,23			
	APA6	669600	685154	677377	1059,83			
8 h	APA7	754593	651093	702843	1099,74	1083,67	2,03	0,19
	APA8	704662	697367	701015	1096,87			
	APA9	683355	664463	673909	1054,39			
12 h	APA10	797200	695316	746258	1167,77	1124,84	50,42	4,48
	APA11	749841	703963	726902	1137,44			
	APA12	701196	665659	683428	1069,31			
24 h	APA13	725099	739381	732240	1145,80	1221,25	142,36	11,66
	APA14	888161	882176	885169	1385,45			
	APA15	747401	700102	723752	1132,50			
48 h	APA16	605007	526617	565812	885,00	815,70	73,42	9,00
	APA17	529826	523114	526470	823,35			
	APA18	490523	454452	472488	738,75			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **A**: ACE buffer solution, **1 – 18**: Sample number.

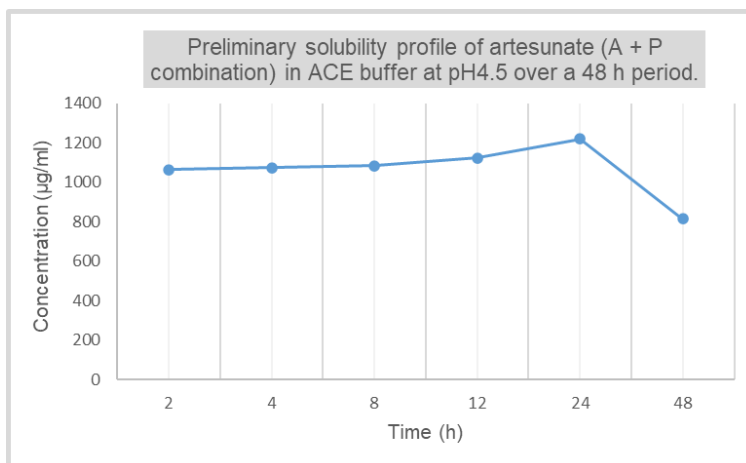


Figure 4.9: Preliminary solubility profile of artesunate (in A + P combination) in ACE buffer solution.

Artesunate in combination with pyrimethamine in the ACE buffer at pH 4.5 obtained equilibrium rapidly and remained stable in solution, where it exhibited a maximum solubility of 1221.25 µg/ml within 24 h and then started to degrade between 24 h and 48 h. The solubility almost doubled when in combination with pyrimethamine upon comparison with the single component. However, due to the clear co-elution seen with the single component, the improvement in solubility couldn't be specifically attributed to the presence of pyrimethamine. Further investigation to optimise the method is needed to distinguish between artesunate and the degradation products. Artesunate proved to be most stable in the ACE buffer solution when in combination with pyrimethamine. ACE buffer at pH 4.5 represents the pH at which artesunate will most likely be 50% ionised: 50% unionised, due to the fact that artesunate has a pKa of 4.63 – close to the pH of ACE buffer ([Al Haydar, 2011](#)). The pH profile for the artesunate single component initially remained constant (approximately the same value as that of the ACE buffer). However, as time progressed and artesunate gradually degraded, the pH decreased to ± 4.3 . This can be due to the formation of succinic acid, which is a degradation product resultant of hydrolysis that possibly occurs in the aqueous media ([Al Haydar, 2011](#)). In contrast, for the A + P combination pH profile, higher pH values were recorded (4.63 as the initial pH and 4.48 at the 48 h time interval), possibly due to the presence of the weakly basic pyrimethamine.

4.3.1.4 Preliminary solubility in potassium phosphate (PBS) buffer solution at pH 6.8

Preliminary solubility results obtained for artesunate (single component) in PBS buffer solution are presented in Table 4.7 and Figure 4.10.

Table 4.7: Preliminary solubility data of artesunate in PBS buffer solution at pH 6.8

Preliminary - Artesunate (Single) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	AP1	363982	363219	363601	559,08	5590,82	5722,80	116,36	2,03
	AP2	376808	379940	378374	581,06	5810,60			
	AP3	373074	377812	375443	576,70	5766,99			
4 h	AP4	334964	336335	335650	517,50	5175,02	5092,00	122,05	2,40
	AP5	318600	322697	320649	495,19	4951,86			
	AP6	336883	330935	333909	514,91	5149,13			
8 h	AP7	234381	233993	234187	366,56	3665,64	3645,08	18,15	0,50
	AP8	233332	231370	232351	363,83	3638,33			
	AP9	231715	232038	231877	363,13	3631,27			
12 h	AP10	144410	146081	145246	234,25	2342,53	2364,59	23,22	0,98
	AP11	144578	148586	146582	236,24	2362,42			
	AP12	146848	149867	148358	238,88	2388,83			
24 h	AP13	97456	177347	137402	222,58	2225,85	1809,19	360,83	19,94
	AP14	96930	93881	95406	160,11	1601,10			
	AP15	95428	95319	95374	160,06	1600,63			
48 h	AP16	25362	21189	23276	52,81	528,09	490,96	56,36	10,77
	AP17	21928	23360	22644	51,87	518,69			
	AP18	16229	16612	16421	42,61	426,11			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: PBS buffer solution, **1 – 18**: Sample number.

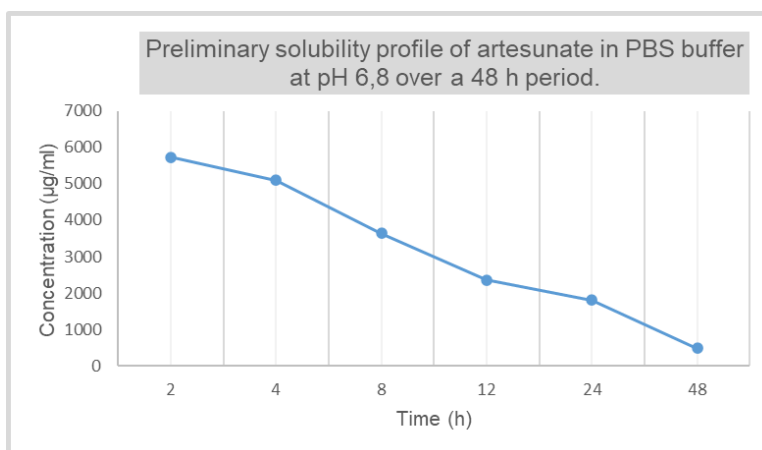


Figure 4.10: Preliminary solubility profile of artesunate (single component) in PBS buffer solution.

From table 4.7, it is clear that artesunate reached a maximum solubility of 5722.80 µg/ml at 2 h, after which the degradation was linear as time evolved. Note that the %RSD is below 5% for 2 – 12 h time intervals, but for 24 h and 48 h it is above 10%. This can probably be attributed to the increasing degradation rate, but it will have to be confirmed with further studies.

Preliminary solubility results obtained for artesunate (A + P combination) in PBS buffer solution are presented in Table 4.8 and Figure 4.11.

Table 4.8: Preliminary solubility data of artesunate in the A+P combination in PBS buffer solution at pH 6.8

Preliminary - Artesunate (A+P combination) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APP1	493736	502019	497878	758,84	7588,35	7292,88	260,09	3,57
	APP2	480095	462338	471217	719,17	7191,74			
	APP3	467378	462528	464953	709,86	7098,56			
4 h	APP4	364911	368677	366794	563,83	5638,33	5676,10	91,79	1,62
	APP5	377544	375192	376368	578,08	5780,75			
	APP6	364611	365063	364837	560,92	5609,22			
8 h	APP7	262938	263812	263375	409,99	4099,85	4132,98	36,27	0,88
	APP8	266637	269777	268207	417,17	4171,73			
	APP9	264406	266041	265224	412,73	4127,35			
12 h	APP10	182411	181983	182197	289,22	2892,23	2990,76	96,76	3,24
	APP11	193222	184905	189064	299,44	2994,38			
	APP12	190297	200102	195200	308,57	3085,66			
24 h	APP13	114963	115884	115424	189,89	1898,90	1998,68	102,80	5,14
	APP14	135191	123264	129228	210,42	2104,25			
	APP15	130664	112819	121742	199,29	1992,88			
48 h	APP16	33223	31022	32123	65,97	659,70	680,54	24,76	3,64
	APP17	33380	32791	33086	67,40	674,02			
	APP18	33537	37190	35364	70,79	707,91			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **P**: PBS buffer solution, **1 – 18**: Sample number.

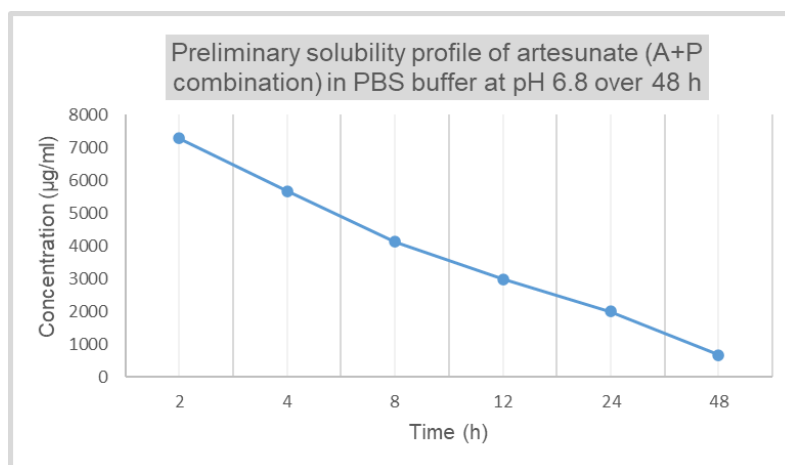


Figure 4.11: Preliminary solubility profile of artesunate (in A + P combination) in PBS buffer solution.

The data analysis indicated that artesunate, when in combination with pyrimethamine, attained a maximum solubility of $\pm 7292 \mu\text{g/ml}$ within 2 h. Thereafter, a linear degradation pattern was observed during the 48 h experiment. Artesunate in combination with pyrimethamine exhibited a higher solubility ($\pm 1500 \mu\text{g/ml}$ more) in the PBS buffer at pH 6.8 compared to the single component. Artesunate ionise better at a high pH ([Al Haydar, 2011](#)), which possibly may be the explanation for the higher solubility when in combination with pyrimethamine; however, the degradation rate seems to be more elevated in combination than in the single component.

The difference in degradation during the 2-4 h time interval was apparent, with a concentration difference of $\pm 630 \mu\text{g/ml}$ for the single component and $\pm 1616 \mu\text{g/ml}$ for the A + P combination. This may suggest that pyrimethamine potentially accelerates the degradation of artesunate. However, further degradation studies are needed.

The initial pH of the solvent was measured at a pH of 6.8. The single component artesunate's pH after 2 h were 5.71, which could be attributed to its acidic nature. Over the course of the 48 h preliminary experiment, the pH further decreased to 4.98, potentially influenced by the degradation product, succinic acid, that contributed to an increase in the acidic nature of the solution. In the A + P combination, artesunate's acidic nature reduced the pH from 6.8 to 5.75 in the initial measurement. Throughout the 48 h the pH decreased only to 5.13, which, notably, remained higher than in the single-component preliminary data. Pyrimethamine appears to possibly mitigate the drastic pH decrease that was observed during the single component's preliminary solubility study, due to its weak basic nature.

4.3.1.5 Preliminary solubility in sodium phosphate (SBS) buffer solution at pH 6.8

Preliminary solubility results obtained for artesunate (single component) in SBS buffer solution are presented in Table 4.9 and Figure 4.12.

Table 4.9: Preliminary solubility data of artesunate in SBS buffer solution at pH 6.8

Preliminary - Artesunate (Single) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	AS1	356951	355776	356364	529,35	5293,51	5326,40	130,39	2,45
	AS2	350129	351939	351034	521,56	5215,61			
	AS3	365399	371486	368443	547,01	5470,08			
4 h	AS4	346970	348880	347925	517,02	5170,16	5159,58	275,71	5,34
	AS5	365893	365487	365690	542,98	5429,85			
	AS6	329066	326910	327988	487,87	4878,73			
8 h	AS7	235971	238006	236989	354,85	3548,52	3515,58	28,52	0,81
	AS8	234140	233061	233601	349,90	3498,99			
	AS9	233431	233804	233618	349,92	3499,24			
12 h	AS10	153825	154678	154252	233,91	2339,08	2316,83	29,21	1,26
	AS11	151683	155255	153469	232,76	2327,65			
	AS12	149518	151414	150466	228,37	2283,75			
24 h	AS13	87460	84706	86083	134,26	1342,61	1313,04	41,82	3,19
	AS14	82457	81617	82037	128,35	1283,47			
	AS15	No data							
48 h	AS16	27891	18983	23437	42,69	426,87	359,59	63,06	17,54
	AS17	17620	18749	18185	35,01	350,09			
	AS18	15543	14223	14883	30,18	301,82			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **S**: SBS buffer solution, **1 – 18**: Sample number.

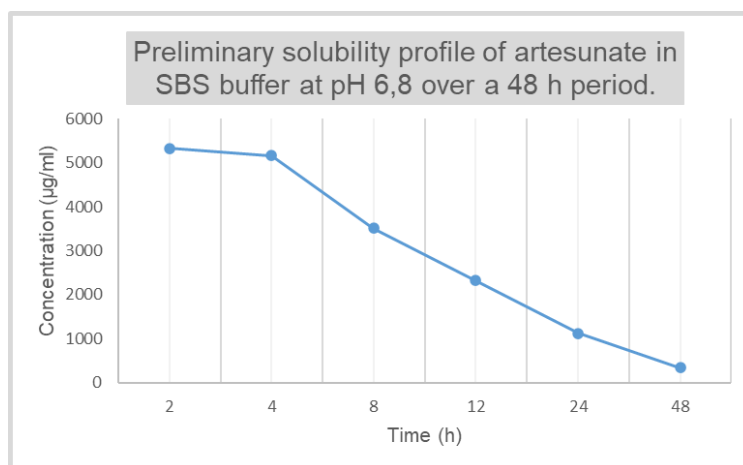


Figure 4.12: Preliminary solubility profile of artesunate (single component) in SBS buffer solution.

Artesunate exhibited a maximum solubility of 5326.40 µg/ml at 2 h as seen from the abovementioned data in Table 4.9. After 2 h the degradation was apparent. Note that in the PBS buffer solution, the degradation between 2 – 4 h was more rapid than in the SBS buffer solution, even though the pH for both buffers are 6.8. The difference in the preliminary solubility profiles can possibly be attributed to the composition of the buffer solutions, since the pH profiles for PBS and SBS at the time intervals were relatively the same.

Preliminary solubility results obtained for artesunate (A + P combination) in SBS buffer solution are presented in Table 4.10 and Figure 4.13.

Table 4.10: Preliminary solubility data of artesunate in the A+P combination in SBS buffer solution at pH 6.8

Preliminary - Artesunate (A+P combination) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APS1	472791	464157	468474	693,23	6932,32	6789,77	135,65	2,00
	APS2	453302	446698	450000	666,23	6662,27			
	APS3	460224	455162	457693	677,47	6774,73			
4 h	APS4	383212	376740	379976	563,87	5638,68	5526,18	99,74	1,80
	APS5	373104	366687	369896	549,13	5491,32			
	APS6	365312	368627	366970	544,85	5448,55			
8 h	APS7	262994	257841	260418	389,10	3891,00	3968,85	105,79	2,67
	APS8	275144	272822	273983	408,93	4089,29			
	APS9	265262	260398	262830	392,63	3926,26			
12 h	APS10	205348	201611	203480	305,87	3058,69	3039,97	358,50	11,79
	APS11	205829	148289	177059	267,25	2672,48			
	APS12	228096	224021	226059	338,87	3388,74			
24 h	APS13	106956	107892	107424	165,46	1654,57	1673,91	24,34	1,45
	APS14	109488	106912	108200	166,59	1665,91			
	APS15	111953	109280	110617	170,12	1701,24			
48 h	APS16	No data					527,90	25,02	4,74
	APS17	29350	28926	29138	51,02	510,20			
	APS18	31417	31701	31559	54,56	545,59			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **A:** Artesunate, **P:** Pyrimethamine, **S:** SBS buffer solution, **1 – 18:** Sample number.

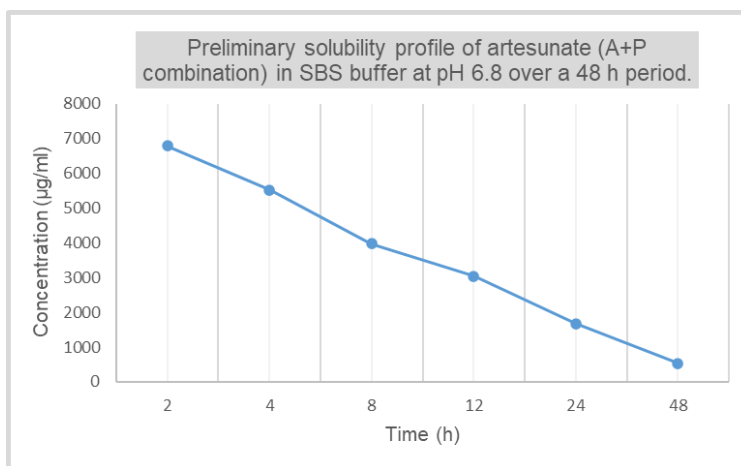


Figure 4.13: Preliminary solubility profile of artesunate (in A + P combination) in SBS buffer solution.

According to the data, artesunate, in the A + P combination, attained a maximum solubility of $\pm 6789 \mu\text{g/ml}$ in 2 h. During the course of the 48 h experiment, a linear degradation was observed. With the presence of pyrimethamine, artesunate is more soluble in the SBS buffer at pH 6.8 than when used alone. It is also possible that artesunate's ionised form is enhanced at a higher pH. Although the solubility increased with pyrimethamine, it appears that the rate of degradation might be higher than with the single component.

With a concentration difference of $\pm 166 \mu\text{g/ml}$ for the single component and $\pm 1263 \mu\text{g/ml}$ for the A + P combination, the degradation differences over the course of the 2-4 h time interval are clearly visible and almost doubled. This implies that pyrimethamine may possibly hasten the degradation of artesunate.

The solvent's initial pH was 6.8, according to the method. Due to its acidic nature, artesunate as the single component had a pH reading of 5.70 after two hours. The pH further decreased to 4.95 during the 48-hour preliminary experiment, possibly as a result of the formation of succinic acid, which raises the solution's acidity. The acidic nature of artesunate lowered the pH in the A + P combination from 6.8 to 5.73 initially. The pH only decreased to 5.11 throughout the course of the 48-hour period, which is somewhat higher than in the single-component preliminary data. Pyrimethamine seems to have the potential to decrease the sudden pH reduction that was seen during the single component experiments, due to its weak basic nature.

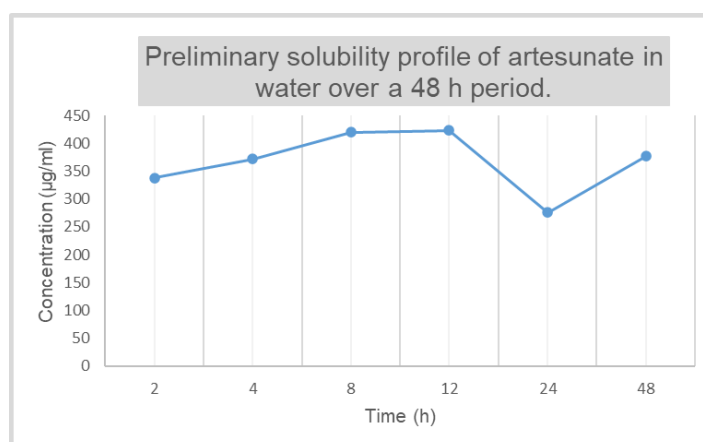
4.3.1.6 Preliminary solubility in water

Preliminary solubility results obtained for artesunate (single component) in ultra-purified water are presented in Table 4.11 and Figure 4.14.

Table 4.11: Preliminary solubility data of artesunate in water

Preliminary - Artesunate (Single) in water								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	AW1	176661	186893	181777	329,78	338,72	8,79	2,59
	AW2	196705	191927	194316	347,34			
	AW3	189496	187268	188382	339,03			
4 h	AW4	223840	212380	218110	380,67	372,55	7,24	1,94
	AW5	210439	210832	210636	370,20			
	AW6	207315	209052	208184	366,77			
8 h	AW7	250896	252109	251503	427,45	420,83	6,07	1,44
	AW8	245622	246051	245837	419,51			
	AW9	242192	243786	242989	415,52			
12 h	AW10	221809	252872	237341	407,61	423,61	17,11	4,04
	AW11	272057	251210	261634	441,64			
	AW12	250216	244391	247304	421,57			
24 h	AW13	148519	148317	148418	283,05	277,12	5,62	2,03
	AW14	144225	143191	143708	276,45			
	AW15	141095	139771	140433	271,87			
48 h	AW16	223050	221498	222274	386,51	377,79	8,82	2,33
	AW17	218737	213647	216192	377,99			
	AW18	211188	208176	209682	368,87			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **W**: water, **1 – 18**: Sample number.

**Figure 4.14:** Preliminary solubility profile of artesunate (single component) in water.

It is clear from Figure 4.14 that artesunate reached a maximum solubility of approximately 420 µg/ml at 8 h and reached equilibrium between 8 h and 12 h, with degradation following at the 24 h time interval. However, there was a spike in concentration at 48 h that could possibly be attributed to a degradation product to cause a co-elution, as seen in the chromatography.

Preliminary solubility results obtained for artesunate (A + P combination) in ultra-purified water are presented in Table 4.12 and Figure 4.15.

Table 4.12: Preliminary solubility data of artesunate in the A+P combination in water

Preliminary - Artesunate (A + P combination) in water								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APW1	1157785	1141496	1149641	1685,54	1681,43	19,70	1,17
	APW2	1131804	1131014	1131409	1660,00			
	APW3	1165604	1152538	1159071	1698,75			
4 h	APW4	1273859	1146392	1210126	1770,26	1601,72	153,10	9,56
	APW5	1058465	1066811	1062638	1563,66			
	APW6	991146	1002163	996655	1471,24			
8 h	APW7	942634	900315	921475	1365,93	1331,90	42,23	3,17
	APW8	909875	903389	906632	1345,14			
	APW9	872521	854356	863439	1284,63			
12 h	APW10	893215	821937	857576	1276,42	1236,34	46,46	3,76
	APW11	822330	851075	836703	1247,18			
	APW12	814722	770489	792606	1185,41			
24 h	APW13	733884	765434	749659	1125,25	1141,93	28,63	2,51
	APW14	737628	762098	749863	1125,54			
	APW15	759375	810957	785166	1174,99			
48 h	APW16	553144	568218	560681	860,54	947,83	109,70	11,57
	APW17	534458	660362	597410	911,99			
	APW18	738294	683511	710903	1070,96			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering: A:** Artesunate, **P:** Pyrimethamine, **W:** water, **1 – 18:** Sample number.

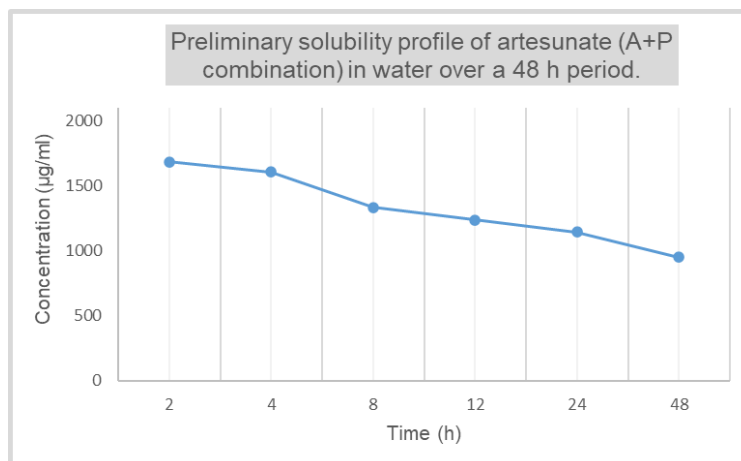


Figure 4.15: Preliminary solubility profile of artesunate (in A + P combination) in water.

From the data obtained, artesunate in combination with pyrimethamine exhibited a maximum solubility of $\pm 1681 \mu\text{g/ml}$ within 2 h after which a linear degradation was apparent. The solubility improved with approximately $1300 \mu\text{g/ml}$, which is triple the solubility of artesunate as a single component.

Although the solubility showed a great improvement in the combination, the data obtained for the artesunate single component seems to be more stable over the 48 h than that observed with the A + P combination.

After 2 h, the pH for artesunate (the single component) was 3.65 due to the very acidic nature of the API. However, it further decreased to 2.23, possibly as a result of the succinic acid, which increased the acidity of the solution even more. The pH of the A + P combination measured within 2 hours was 4.92. During the 48-hour period, the pH decreased to 4.37, which is significantly higher than in the single-component preliminary data.

4.3.1.7 Pivot solubility of artesunate single component and artesunate in A + P combination

In Table 4.13 and Figure 4.16 below, is a compilation of the pivot solubility studies performed for artesunate as a single component and in combination with pyrimethamine in the various solubility media ranging from a pH 1.2 – 6.8. Please note that due to artesunate's instability in the various media, it was decided that withdrawal for the pivot solubility studies would be performed at 2 hours.

Table 4.13: Pivot solubility of artesunate in single component and in combination with pyrimethamine in various media

Solvent	Average Concentration ($\mu\text{g/ml}$)					
	ART (single)	SD	%RSD	(A+P Combo)	SD	%RSD
0.1 N HCl	182,71	57,45	31,45	0,00	0,00	0,00
NaCl	200,05	69,14	34,56	232,27	47,97	20,65
ACE	778,19	20,44	2,63	1041,50	118,89	11,42
PBS	6859,16	9,69	0,14	7506,60	50,47	0,67
SBS	6636,06	13,54	3,19	6776,78	14,85	0,22
Water	438,66	15,41	3,51	1718,29	36,49	2,12

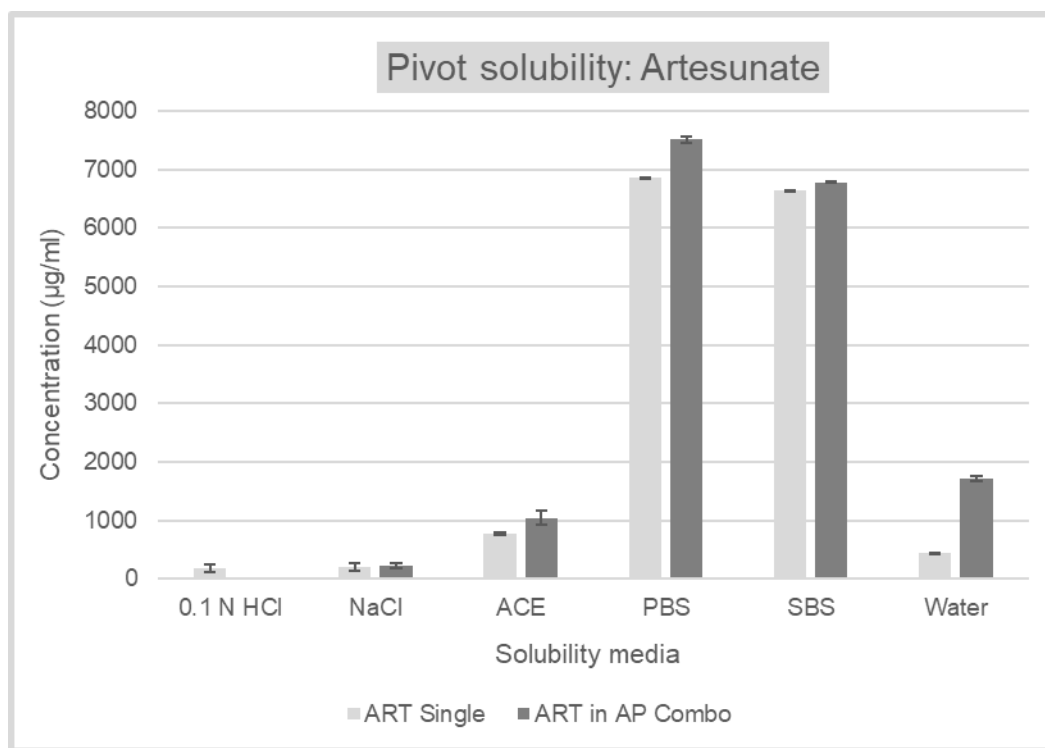


Figure 4.16: Pivot solubility of artesunate in single component and A + P combination in various media.

The data presented in Figure 4.16 and Table 4.13 clearly indicate that the solubility of artesunate (both as a single component and in combination with pyrimethamine) increased as the pH of the various media increased. When considering the solubility of artesunate as a single component, a lower solubility was obtained in all the media compared to artesunate in

combination with pyrimethamine, with the exception of artesunate in combination with pyrimethamine in the 0.1 N HCl buffer. Since no detectable chromatography was obtained for the combination in this solvent, the data could not be analysed. This could possibly be due to degradation and should be further investigated to establish the reason for this event.

The solubility data for artesunate as a single component compared to the combination produced similar results in both the NaCl and SBS buffer solutions. In potassium phosphate (PBS) buffer, both the single component and artesunate in combination displayed the highest solubility compared to all of the solubility media.

Furthermore, it is noteworthy that the most substantial increase in artesunate's solubility was when combined with pyrimethamine in water, where the increase exceeded three-fold in comparison to its solubility as a single component.

4.3.2 Pyrimethamine

In this section, pyrimethamine as a single component, the artesunate-pyrimethamine combination, and the pyrimethamine-sulfadoxine combination are discussed. Preliminary solubility studies were performed in water and various media ranging from pH 1.2 – 6.8. In all preliminary experiments, the pH of the solvents was measured before initiating the experiment. Then, the pH of the solution (solute + solvent) was taken after the various time intervals.

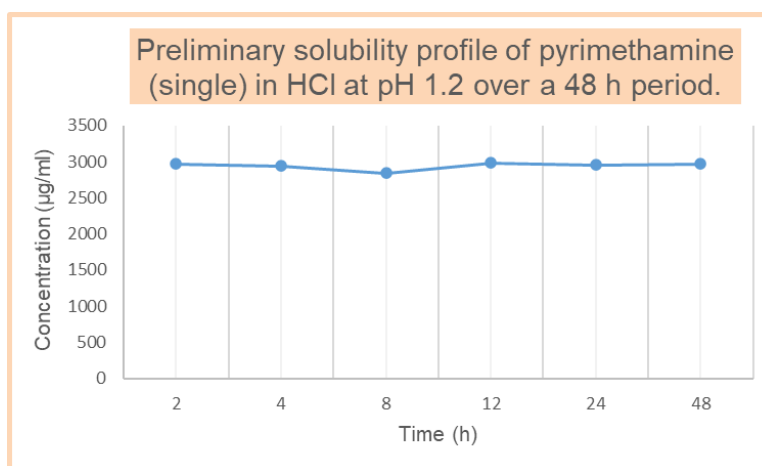
4.3.2.1 Preliminary solubility in 0.1 N HCl solution at pH 1.2

Preliminary solubility results obtained for pyrimethamine (single component) in 0.1 N HCl solution are presented in Table 4.14 and Figure 4.17.

Table 4.14: Preliminary solubility data of pyrimethamine in 0.1 N HCl at pH 1.2

Preliminary - Pyrimethamine (Single) in HCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	PH1	681063	678631	679847	29,60	2959,84	2968,35	70,59	2,38
	PH2	665922	667371	666647	29,02	2902,4			
	PH3	697291	700543	698917	30,43	3042,82			
4 h	PH4	668306	666711	667509	29,06	2906,15	2941,85	42,53	1,45
	PH5	672065	674144	673105	29,31	2930,5			
	PH6	687302	685751	686527	29,89	2988,91			
8 h	PH7	682919	687074	684997	29,82	2982,25	2841,43	193,82	6,82
	PH8	669674	672475	671075	29,22	2921,67			
	PH9	602207	601453	601830	26,20	2620,37			
12 h	PH10	686697	683386	685042	29,82	2982,44	2981,37	37,71	1,26
	PH11	674356	677660	676008	29,43	2943,14			
	PH12	691314	695361	693338	30,19	3018,54			
24 h	PH13	659661	663879	661770	28,81	2881,18	2956,74	71,55	2,42
	PH14	692550	696383	694467	30,23	3023,45			
	PH15	681576	680756	681166	29,66	2965,58			
48 h	PH16	683345	684479	683912	29,78	2977,53	2971,18	5,50	0,19
	PH17	679422	684056	681739	29,68	2968,07			
	PH18	680013	683397	681705	29,68	2967,93			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **P**: Pyrimethamine, **H**: HCl solution, **1 – 18**: Sample number.

**Figure 4.17:** Preliminary solubility profile of pyrimethamine (single component) in 0.1 N HCl solution.

From Figure 4.17, it is evident that pyrimethamine attained its maximal solubility of approximately 2981 µg/ml within 12 h. However, from 2 h to 48 h there was little variation in the concentration at each interval. Therefore, it can be said that pyrimethamine went quickly into solution and remained stable and in equilibrium in the 0.1 N HCl solution over the 48 h period.

The preliminary solubility results obtained for pyrimethamine (in A + P combination) in 0.1 N HCl solution are presented in Table 4.15 and Figure 4.18.

Table 4.15: Preliminary solubility data of pyrimethamine in the A+P combination in 0.1 N HCl at pH 1.2

Preliminary - Pyrimethamine (A+P combination) in HCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APH1	710704	710319	710512	30,93	3093,27	2936,67	137,78	4,69
	APH2	652091	649781	650936	28,34	2834,04			
	APH3	660709	663528	662119	28,83	2882,70			
4 h	APH4	645491	661419	653455	28,45	2845,00	2928,83	95,91	3,27
	APH5	689164	646737	667951	29,08	2908,08			
	APH6	696790	696722	696756	30,33	3033,42			
8 h	APH7	691475	696339	693907	30,21	3021,02	3098,73	76,15	2,46
	APH8	728446	729329	728888	31,73	3173,23			
	APH9	712379	712626	712503	31,02	3101,93			
12 h	APH10	725446	727469	726458	31,63	3162,66	3189,89	45,82	1,44
	APH11	722982	730659	726821	31,64	3164,23			
	APH12	747603	742144	744874	32,43	3242,79			
24 h	APH13	708900	710463	709682	30,90	3089,66	3106,22	60,36	1,94
	APH14	734377	723357	728867	31,73	3173,14			
	APH15	704950	698883	701917	30,56	3055,87			
48 h	APH16	843357	846006	844682	36,77	3677,08	3313,99	316,85	9,56
	APH17	712030	709067	710549	30,93	3093,43			
	APH18	728353	728612	728483	31,71	3171,47			

***AUC**: Area under the curve, **AVG**: Average area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **H**: HCl solution, **1 – 18**: Sample number.

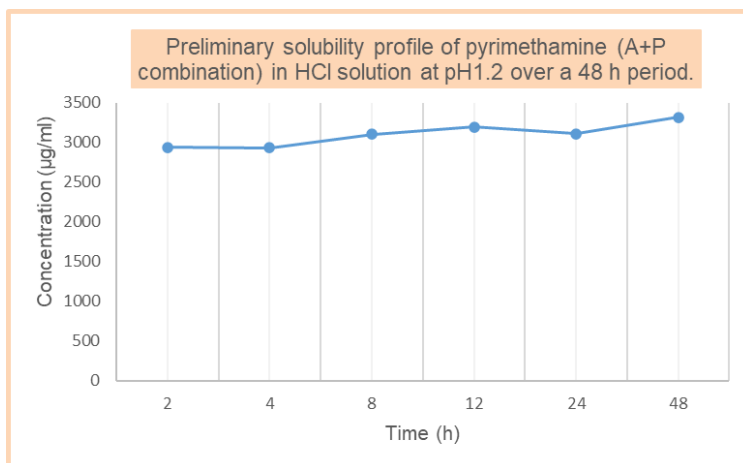


Figure 4.18: Preliminary solubility profile of pyrimethamine (in A + P combination) in 0.1 N HCl solution.

From the acquired data, pyrimethamine exhibited rapid solubility, stability, and sustained equilibrium in solution. Over 48 h, the solubility gradually improved, with a maximum solubility of $\pm 3313 \mu\text{g/ml}$ at 48 h compared to the single component, which displayed a steady solubility profile throughout the 48 h experiment.

The preliminary solubility results obtained for pyrimethamine (in S + P combination) in 0.1 N HCl solution are presented in Table 4.16 and Figure 4.19.

Table 4.16: Preliminary solubility data of pyrimethamine in the S+P combination in 0.1 N HCl at pH 1.2

Preliminary - Pyrimethamine (S + P combination) in HCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPH1	444410	411309	427860	18,63	1863,38	1756,45	164,66	9,37
	SPH2	419501	425071	422286	18,39	1839,13			
	SPH3	353121	366290	359706	15,67	1566,83			
4 h	SPH4	543363	538670	541017	23,56	2355,76	2357,61	57,88	2,46
	SPH5	555936	553973	554955	24,16	2416,4			
	SPH6	505845	550873	528359	23,01	2300,68			
8 h	SPH7	595050	547912	571481	24,88	2488,32	2502,92	270,61	10,81
	SPH8	535092	493695	514394	22,40	2239,91			
	SPH9	645299	631980	638640	27,81	2780,54			
12 h	SPH10	653579	653062	653321	28,44	2844,42	2828,77	258,51	9,14
	SPH11	603819	573373	588596	25,63	2562,79			
	SPH12	705735	708772	707254	30,79	3079,09			
24 h	SPH13	561593	513654	537624	23,41	2340,99	2489,14	140,78	5,66
	SPH14	602279	548466	575373	25,05	2505,25			
	SPH15	574336	629693	602015	26,21	2621,17			
48 h	SPH16	566409	558015	562212	24,48	2447,98	2573,42	300,05	11,66
	SPH17	640956	698508	669732	29,16	2915,83			
	SPH18	545249	537098	541174	23,56	2356,44			

***AUC**: Area under the curve, **AVG**: Average area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **P**: Pyrimethamine, **H**: HCl solution, **1 – 18**: Sample number.

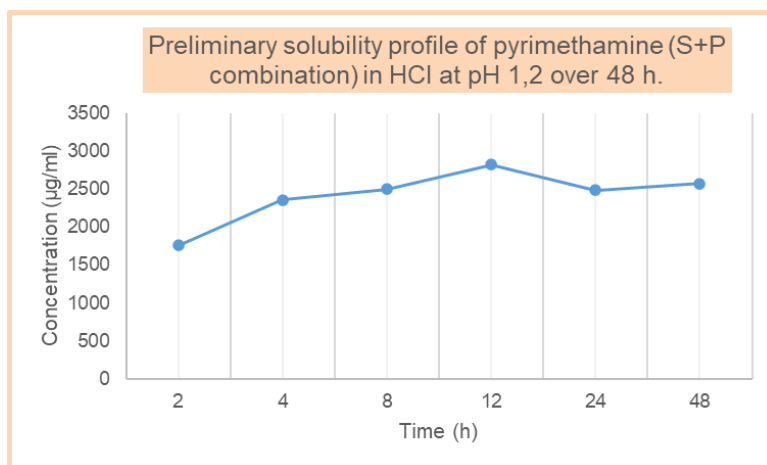


Figure 4.19: Preliminary solubility profile of pyrimethamine (in S + P combination) in 0.1 N HCl solution.

Pyrimethamine dissolved gradually, where maximal solubility was attained within 12 h with a concentration of 2820 µg/ml. The solubility of pyrimethamine in the presence of sulfadoxine appeared to be lower (± 148 µg/ml less) than in comparison to the single component.

4.3.2.2 Preliminary solubility in NaCl buffer solution at pH 1.2

Preliminary solubility results obtained for pyrimethamine (single component) in NaCl buffer solution are presented in Table 4.17 and Figure 4.20.

Table 4.17: Preliminary solubility data of pyrimethamine in NaCl buffer solution at pH 1.2

Preliminary - Pyrimethamine (Single) in NaCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	PN1	549927	551309	550618	23,15	2315,43	2366,34	45,35	1,92
	PN2	563463	569224	566344	23,81	2381,20			
	PN3	569774	573048	571411	24,02	2402,39			
4 h	PN4	545580	541412	543496	22,86	2285,64	2278,96	47,44	2,08
	PN5	530880	528805	529843	22,29	2228,54			
	PN6	546862	557856	552359	23,23	2322,71			
8 h	PN7	566333	573590	569962	23,96	2396,33	2329,48	59,15	2,54
	PN8	549169	548611	548890	23,08	2308,20			
	PN9	542527	543639	543083	22,84	2283,92			
12 h	PN10	549730	547322	548526	23,07	2306,68	2270,32	51,81	2,28
	PN11	526011	525286	525649	22,11	2211,00			
	PN12	544782	545855	545319	22,93	2293,27			
24 h	PN13	569052	569920	569486	23,94	2394,34	2378,74	13,52	0,57
	PN14	562467	565403	563935	23,71	2371,13			
	PN15	564320	563366	563843	23,71	2370,74			
48 h	PN16	No data					1658,33	82,28	4,96
	PN17	376620	382568	379594	16,00	1600,15			
	PN18	380620	434209	407415	17,17	1716,51			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **Fin. Conc:** Final Concentration, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **P:** Pyrimethamine, **N:** NaCl buffer solution, **1 – 18:** Sample number.

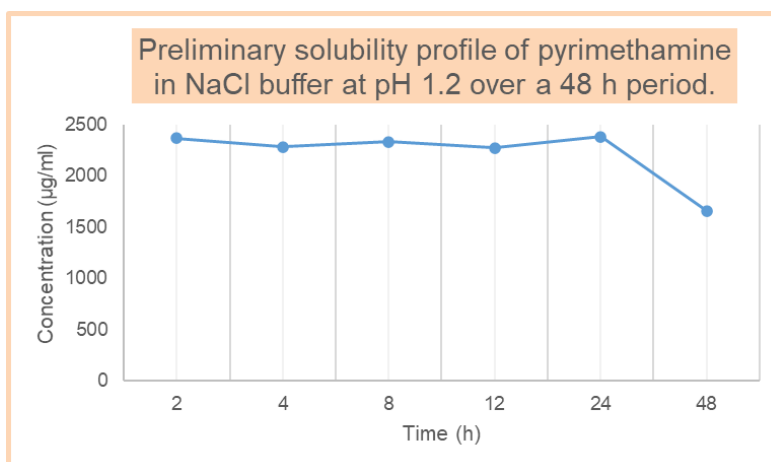


Figure 4.20: Preliminary solubility profile of pyrimethamine (single component) in NaCl buffer solution.

From Figure 4.20, it can be seen that pyrimethamine had reached a maximum solubility of approximately 2378 µg/ml within 24 h. The variance in concentration between each time interval was very low; thus, it can be said that pyrimethamine dissolves rapidly and stays in equilibrium for the whole 24 h period, after which degradation is seen at 48 h.

Preliminary solubility results obtained for pyrimethamine (A + P combination) in NaCl buffer solution are presented in Table 4.18 and Figure 4.21.

Table 4.18: Preliminary solubility data of pyrimethamine in the A + P combination in NaCl buffer solution at pH 1.2

Preliminary - Pyrimethamine (A + P combination) in NaCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APN1	557738	550904	554321	23,31	2330,92	2326,53	15,75	0,68
	APN2	561265	551535	556400	23,40	2339,61			
	APN3	548285	549900	549093	23,09	2309,05			
4 h	APN4	591675	590902	591289	24,86	2485,53	2396,85	78,34	3,27
	APN5	555101	556479	555790	23,37	2337,06			
	APN6	566471	559886	563179	23,68	2367,96			
8 h	APN7	579988	577839	578914	24,34	2433,77	2424,55	45,37	1,87
	APN8	586839	585734	586287	24,65	2464,61			
	APN9	565754	564103	564929	23,75	2375,28			
12 h	APN10	613185	608076	610631	25,66	2566,42	2472,19	81,86	3,31
	APN11	576147	574424	575286	24,19	2418,60			
	APN12	578519	578244	578382	24,32	2431,55			
24 h	APN13	572464	569889	571177	24,01	2401,41	2448,03	65,93	2,69
	APN14	593996	592947	593472	24,95	2494,66			
	APN15	No data							
48 h	APN16	545502	545294	545398	22,94	2293,60	2259,99	52,71	2,33
	APN17	545072	542638	543855	22,87	2287,14			
	APN18	521441	524233	522837	21,99	2199,24			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **N**: NaCl buffer solution, **1 – 18**: Sample number.

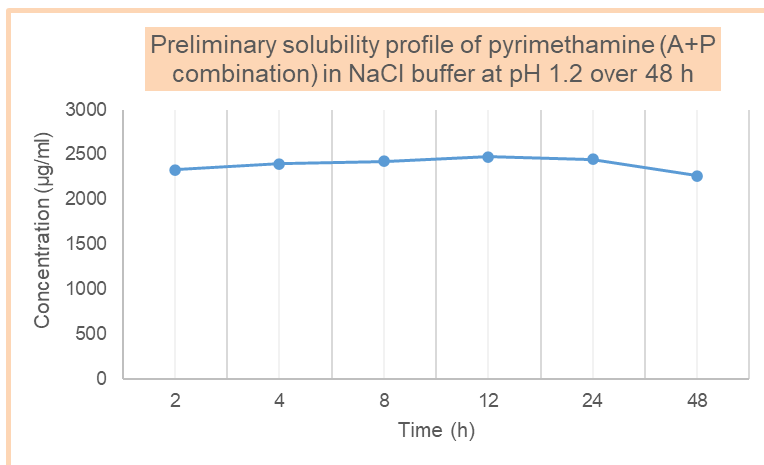


Figure 4.21: Preliminary solubility profile of pyrimethamine (in A + P combination) in NaCl buffer solution.

From the acquired data, it is evident that pyrimethamine exhibited rapid solubility, stability, and sustained equilibrium in solution in the 48 h experiment. The maximum solubility of pyrimethamine (in A + P combination) was $\pm 2472 \mu\text{g/ml}$ at 12 h. In contrast to the single component, the A + P combination demonstrated a more stable solubility profile for the entire 48 h study.

The preliminary solubility results obtained for pyrimethamine (S + P combination) in NaCl buffer solution are presented in Table 4.19 and Figure 4.22.

Table 4.19: Preliminary solubility data of pyrimethamine in the S+P combination in NaCl buffer solution at pH 1.2

Preliminary - Pyrimethamine (S + P combination) in NaCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. ($\mu\text{g/ml}$)	AVG. Conc. ($\mu\text{g/ml}$)	SD	%RSD
2 h	SPN1	No data					2050,39	224,49	10,95
	SPN2	469577	429007	449292	18,92	1891,65			
	SPN3	507459	542941	525200	22,09	2209,12			
4 h	SPN4	505577	499084	502331	21,13	2113,48	2245,45	296,45	13,20
	SPN5	614322	615809	615066	25,85	2584,97			
	SPN6	465824	502701	484263	20,38	2037,91			
8 h	SPN7	518966	565238	542102	22,80	2279,81	2158,24	266,08	12,33
	SPN8	440449	439692	440071	18,53	1853,08			
	SPN9	560903	552950	556927	23,42	2341,81			
12 h	SPN10	518112	516271	517192	21,76	2175,63	2273,18	199,38	8,77
	SPN11	505624	512374	508999	21,41	2141,37			
	SPN12	595990	594727	595359	25,03	2502,55			
24 h	SPN13	624478	626261	625370	26,28	2628,07	2500,65	384,56	15,38
	SPN14	490795	492386	491591	20,69	2068,56			
	SPN15	668075	667433	667754	28,05	2805,33			
48 h	SPN16	545295	596960	571128	24,01	2401,21	2365,65	42,65	1,80
	SPN17	551238	551399	551319	23,18	2318,36			
	SPN18	541429	589436	565433	23,77	2377,39			

***AUC:** Area under the curve, **AVG:** Average area under the curve, **Conc:** Concentration in $\mu\text{g/ml}$, **Fin. Conc.:** Final concentration, **AVG. Conc:** Average concentration in $\mu\text{g/ml}$, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **S:** Sulfadoxine, **P:** Pyrimethamine, **N:** NaCl buffer solution, **1 – 18:** Sample number.

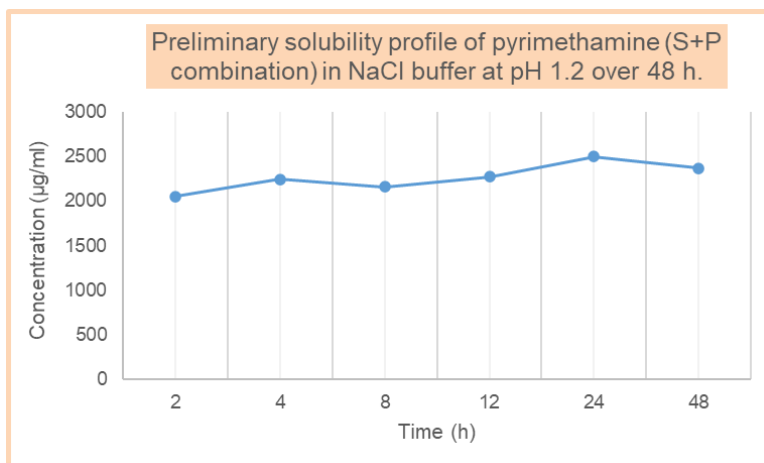


Figure 4.22: Preliminary solubility profile of pyrimethamine (in S + P combination) in NaCl buffer solution.

Pyrimethamine exhibited a gradual increase in solubility, reaching a maximum solubility of $\pm 2500 \mu\text{g/ml}$, as seen in Table 4.18 within 24 h, followed by a very slight decrease after 48 h. The solubility appears to be the same as in comparison to pyrimethamine in the A + P combination.

4.3.2.3 Preliminary solubility in acetate (ACE) buffer solution at pH 4.5

Preliminary solubility results obtained for pyrimethamine (single component) in ACE buffer solution are presented in Table 4.20 and Figure 4.23.

Table 4.20: Preliminary solubility data of pyrimethamine in ACE buffer solution at pH 4.5

Preliminary - Pyrimethamine (Single) in ACE									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	PA1	875957	882257	879107	37,45	3744,59	3762,44	76,19	2,02
	PA2	867044	868338	867691	36,97	3696,75			
	PA3	902249	904340	903295	38,46	3845,96			
4 h	PA4	898481	899965	899223	38,29	3828,90	3863,89	38,89	1,01
	PA5	917857	917274	917566	39,06	3905,77			
	PA6	905959	905899	905929	38,57	3857,00			
8 h	PA7	874095	872658	873377	37,21	3720,58	3818,44	84,78	2,22
	PA8	907700	910135	908918	38,70	3869,52			
	PA9	905686	910091	907889	38,65	3865,21			
12 h	PA10	905384	905362	905373	38,55	3854,67	3901,79	75,01	1,92
	PA11	938829	935683	937256	39,88	3988,28			
	PA12	905753	908691	907222	38,62	3862,42			
24 h	PA13	918174	926860	922517	39,27	3926,52	3923,72	17,18	0,44
	PA14	921405	929741	925573	39,39	3939,32			
	PA15	918651	916263	917457	39,05	3905,31			
48 h	PA16	907586	915837	911712	38,81	3881,23	3868,67	40,74	1,05
	PA17	912117	921053	916585	39,02	3901,66			
	PA18	894234	901460	897847	38,23	3823,13			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering: P**: Pyrimethamine, **A**: ACE buffer solution, **1 – 18**: Sample number.

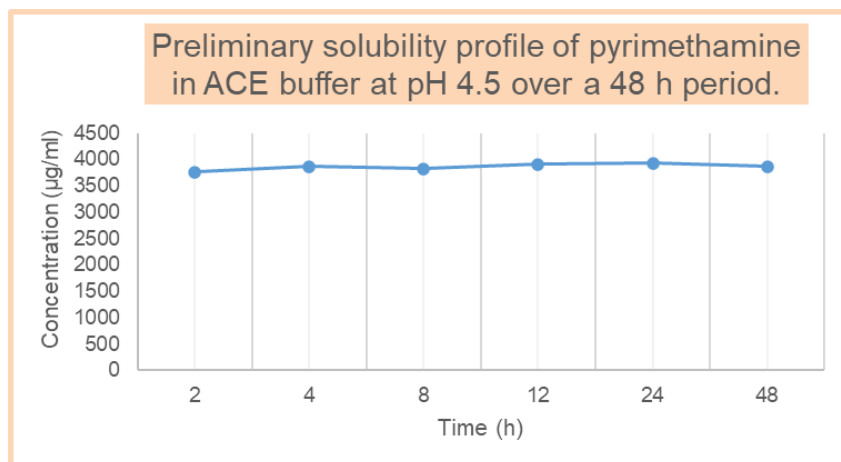


Figure 4.23: Preliminary solubility profile of pyrimethamine (single component) in ACE buffer solution.

From the experiments, it is evident that pyrimethamine went into solution rapidly (at 2 h), and maintained equilibrium for 48 h. Therefore, it can be inferred that pyrimethamine is stable in ACE buffer. From the data in Table 4.20, the maximum solubility was $\pm 3923 \mu\text{g/ml}$ at 24 h, and the variation in concentration between each time sample was low.

Preliminary solubility results obtained for pyrimethamine (A + P combination) in ACE buffer solution are presented in Table 4.21 and Figure 4.24.

Table 4.21: Preliminary solubility data of pyrimethamine in the A+P combination in ACE buffer solution at pH 4.5

Preliminary - Pyrimethamine (A + P combination) in ACE									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. ($\mu\text{g/ml}$)	AVG. Conc. ($\mu\text{g/ml}$)	SD	%RSD
2 h	APA1	725760	722545	724153	30,95	3095,22	3143,66	60,88	1,94
	APA2	730082	731852	730967	31,24	3123,77			
	APA3	752575	751466	752021	32,12	3212,00			
4 h	APA4	803628	806381	805005	34,34	3434,05	3488,50	73,19	2,10
	APA5	809946	812328	811137	34,60	3459,75			
	APA6	838105	837595	837850	35,72	3571,70			
8 h	APA7	918020	923407	920714	39,19	3918,96	3762,64	147,08	3,91
	APA8	849707	852380	851044	36,27	3626,99			
	APA9	878238	878729	878484	37,42	3741,98			
12 h	APA10	895075	905296	900186	38,33	3832,93	3860,13	32,99	0,85
	APA11	914539	916325	915432	38,97	3896,82			
	APA12	902747	906074	904411	38,51	3850,64			
24 h	APA13	928120	936854	932487	39,68	3968,30	3898,25	75,55	1,94
	APA14	897027	896308	896668	38,18	3818,19			
	APA15	914695	921624	918160	39,08	3908,25			
48 h	APA16	943946	944082	944014	40,17	4016,60	4094,56	79,32	1,94
	APA17	973479	968085	970782	41,29	4128,78			
	APA18	968982	977113	973048	41,38	4138,28			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in $\mu\text{g/ml}$, **AVG. Conc:** Average concentration in $\mu\text{g/ml}$, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **A:** Artesunate, **P:** Pyrimethamine, **A:** ACE buffer solution, **1 – 18:** Sample number.

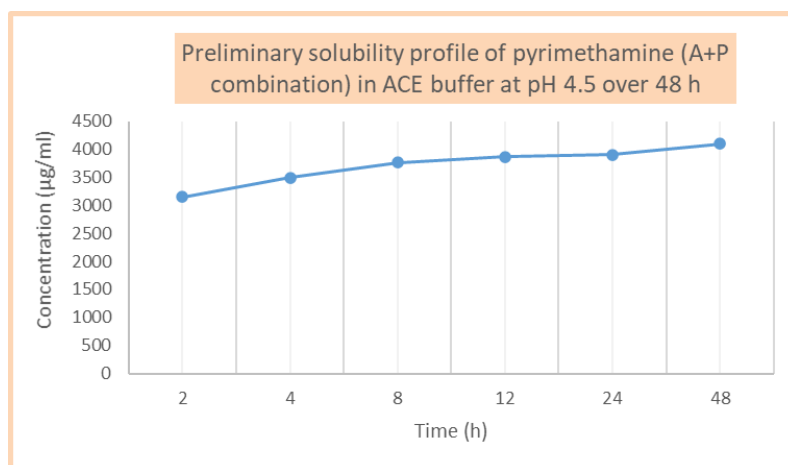


Figure 4.24: Preliminary solubility profile of pyrimethamine (in A + P combination) in ACE buffer solution.

From the acquired data, pyrimethamine exhibited rapid solubility, stability, and sustained equilibrium in solution. Over 48 h, the solubility gradually improved, with a maximum solubility of $\pm 4094 \mu\text{g/ml}$ at 48 h compared to the single component, which displayed a steady solubility profile throughout the 48 h experiment.

The preliminary solubility results obtained for pyrimethamine (the S + P combination) in ACE buffer solution are presented in Table 4.22 and Figure 4.25.

Table 4.22: Preliminary solubility data of pyrimethamine in the S+P combination in ACE buffer at pH 4.5

Preliminary - Pyrimethamine (S + P combination) in ACE									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPA1	996794	1014168	1005481	42,74	4274,2	4311,26	119,13	2,76
	SPA2	990643	992093	991368	42,15	4215,05			
	SPA3	1043844	1048398	1046121	44,45	4444,51			
4 h	SPA4	1041262	1044217	1042740	44,30	4430,34	4409,24	29,84	0,68
	SPA5	No data							
	SPA6	1012923	1052414	1032669	43,88	4388,14			
8 h	SPA7	1017314	999084	1008199	42,86	4285,59	4370,39	73,70	1,69
	SPA8	1032515	1041649	1037082	44,07	4406,63			
	SPA9	1034482	1045561	1040022	44,19	4418,95			
12 h	SPA10		1221984	1221984	51,82	5181,51	4676,98	439,98	9,41
	SPA11	1055435	1052006	1053721	44,76	4476,36			
	SPA12	1021816	1036332	1029074	43,73	4373,07			
24 h	SPA13	957749	975777	966763	41,12	4111,94	4237,23	136,85	3,23
	SPA14	1029699	1033318	1031509	43,83	4383,27			
	SPA15	992574	990842	991708	42,16	4216,48			
48 h	SPA16	948726	963784	956255	40,68	4067,9	4247,95	199,50	4,70
	SPA17	1023172	1023981	1023577	43,50	4350,03			
	SPA18	1018814	1016827	1017821	43,26	4325,91			

***AUC:** Area under the curve, **AVG:** Average area under the curve, **Conc:** Concentration in µg/ml, **Fin. Conc.:** Final concentration, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **S:** Sulfadoxine, **P:** Pyrimethamine, **A:** ACE buffer solution, **1 – 18:** Sample number.

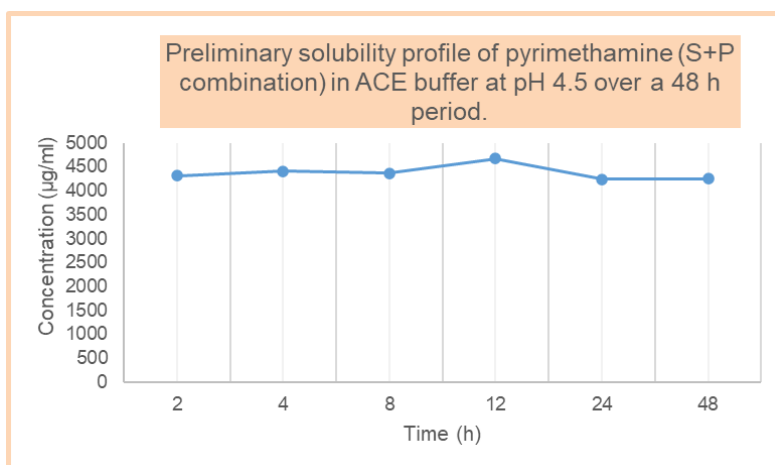


Figure 4.25: Preliminary solubility profile of pyrimethamine (in S + P combination) in ACE buffer solution.

The experimental results clearly indicate that pyrimethamine rapidly dissolved within 2 h and maintained equilibrium throughout the 48 h study. This suggests the stability of pyrimethamine was maintained in the presence of sulfadoxine within the ACE buffer at pH 4.5. From the data in Table 4.21, a maximum solubility of approximately 4676 µg/ml was attained within 24 h, which is an improvement of approximately 1000 µg/ml. To address the high variation observed in the 4 h interval, an adjusted average concentration was calculated, excluding sample “SPA5.” It is important to note that the findings are only part of the preliminary studies, and as such, the decision to discard the aforementioned sample was deemed acceptable. Additionally, for sample “SPA10,” only one AUC was obtained due to logistical difficulties.

4.3.2.4 Preliminary solubility in potassium phosphate (PBS) buffer solution at pH 6.8

Preliminary solubility results obtained for pyrimethamine (single component) in PBS buffer solution are presented in Table 4.23 and Figure 4.26.

Table 4.23: Preliminary solubility data of pyrimethamine in PBS buffer solution at pH 6.8

Preliminary - Pyrimethamine (Single) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	PP1	127193	114102	120648	5,95	65,49	67,50	2,75	4,07
	PP2	130577	114647	122612	6,03	66,38			
	PP3	131649	132554	132102	6,42	70,63			
4 h	PP4	123763	124722	124243	6,10	67,11	66,93	0,75	1,12
	PP5	127024	123528	125276	6,14	67,57			
	PP6	127566	116471	122019	6,01	66,11			
8 h	PP7	132214	129611	130913	6,37	70,10	68,63	2,55	3,72
	PP8	131328	130513	130921	6,37	70,10			
	PP9	125707	116406	121057	5,97	65,68			
12 h	PP10	122699	110218	116459	5,78	63,61	66,85	2,84	4,25
	PP11	127014	129563	128289	6,27	68,92			
	PP12	126140	126386	126263	6,18	68,01			
24 h	PP13	No data					72,93	3,13	4,29
	PP14	142011	142274	142143	6,83	75,14			
	PP15	134447	130106	132277	6,43	70,71			
48 h	PP16	123959	124360	124160	6,10	67,07	67,76	0,60	0,88
	PP17	128323	124408	126366	6,19	68,06			
	PP18	126259	126829	126544	6,19	68,14			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **P**: Pyrimethamine, **P**: PBS buffer solution, **1 – 18**: Sample number.

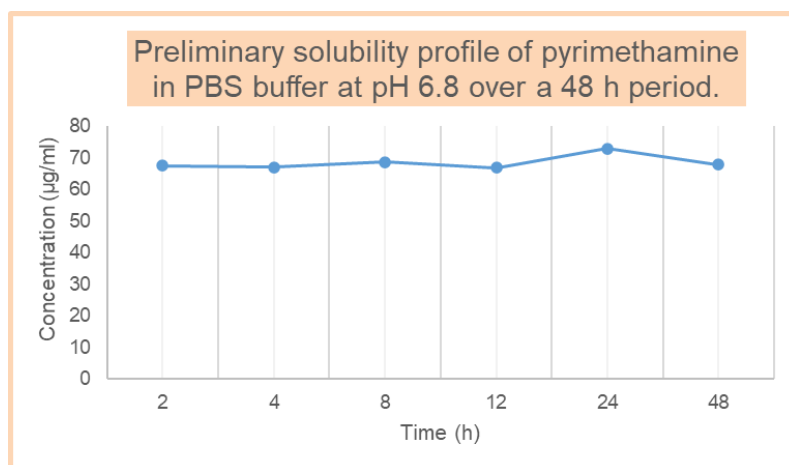


Figure 4.26: Preliminary solubility profile of pyrimethamine (single component) in PBS buffer solution.

It is clear that pyrimethamine rapidly reached its maximum solubility and remained stable in solution during the 48 hours. Its maximal solubility was approximately 72 µg/ml, but early in the experiment, equilibrium was attained and maintained.

Preliminary solubility results obtained for pyrimethamine (A + P combination) in PBS buffer solution are presented in Table 4.24 and Figure 4.27.

Table 4.24: Preliminary solubility data of pyrimethamine in the A+P combination in PBS buffer solution at pH 6.8

Preliminary - Pyrimethamine (A + P combination) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APP1	91963	91923	91943	4,78	478,29	476,23	5,06	1,06
	APP2	91067	93623	92345	4,80	479,93			
	APP3	90203	89846	90025	4,70	470,47			
4 h	APP4	124286	113634	118960	5,89	588,52	588,69	6,67	1,13
	APP5	119685	115090	117388	5,82	582,10			
	APP6	118220	123097	120659	5,95	595,45			
8 h	APP7	169145	168925	169035	7,93	792,81	774,39	22,19	2,87
	APP8	159753	157212	158483	7,50	749,76			
	APP9	163184	168898	166041	7,81	780,60			
12 h	APP10	212171	214305	213238	9,73	973,15	1014,10	35,52	3,50
	APP11	230150	227449	228800	10,37	1036,64			
	APP12	227840	227732	227786	10,33	1032,50			
24 h	APP13	181986	185601	183794	8,53	853,02	951,78	57,02	5,99
	APP14	206531	209186	207859	9,51	951,20			
	APP15	208137	208144	208141	9,52	952,35			
48 h	APP16	184532	187371	185952	8,62	861,83	922,66	54,22	5,88
	APP17	205514	204834	205174	9,40	940,25			
	APP18	214376	208550	211463	9,66	965,91			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **P**: PBS buffer solution, **1 – 18**: Sample number.

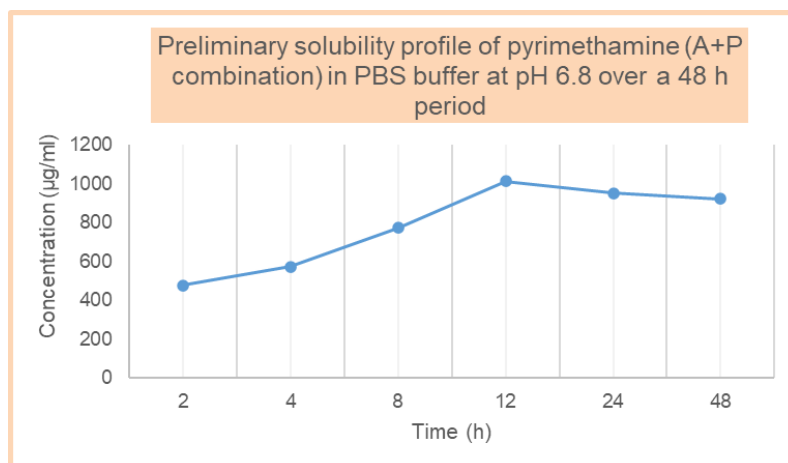


Figure 4.27: Preliminary solubility profile of pyrimethamine (in A + P combination) in PBS buffer solution.

From the acquired data, pyrimethamine in the A + P combination attained an increase in solubility and reached equilibrium at 12 h with a maximum solubility of $\pm 1014 \mu\text{g/ml}$, which is a 14-fold increase compared to the single component profile. However, the improvement cannot only be attributed to the presence of artesunate but could possibly also be due to the degradation of artesunate, which causes a more acidic environment (see subsection 4.3.1.4, page 72), which is ideal for the solubility of pyrimethamine.

The preliminary solubility results obtained for pyrimethamine (in S + P combination) in PBS buffer solution are presented in Table 4.25 and Figure 4.28.

Table 4.25: Preliminary solubility data of pyrimethamine in the S+P combination in PBS buffer at pH 6.8

Preliminary - Pyrimethamine (S + P combination) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. ($\mu\text{g/ml}$)	AVG. Conc. ($\mu\text{g/ml}$)	SD	%RSD
2 h	SPP1	No data					86,19	5,15	5,98
	SPP2	176546	177524	177035	8,25	82,55			
	SPP3	194053	195734	194894	8,98	89,83			
4 h	SPP4	186676	181172	183924	8,54	85,36	85,87	0,82	0,95
	SPP5	184990	183262	184126	8,54	85,44			
	SPP6	184658	190338	187498	8,68	86,81			
8 h	SPP7	161917	167345	164631	7,75	77,48	83,10	5,43	6,53
	SPP8	183547	175151	179349	8,35	83,49			
	SPP9	190055	192304	191180	8,83	88,32			
12 h	SPP10	171843	171625	171734	8,04	80,38	86,21	5,45	6,32
	SPP11	198436	197940	198188	9,12	91,17			
	SPP12	178981	197292	188137	8,71	87,07			
24 h	SPP13	191816	183768	187792	8,69	86,93	82,14	2,78	3,38
	SPP14	181256	169611	175434	8,19	81,89			
	SPP15	178460	174842	176651	8,24	82,39			
48 h	SPP16	204997	196949	200973	9,23	92,31	86,88	4,93	5,67
	SPP17	175738	179070	177404	8,27	82,70			
	SPP18	179028	190231	184630	8,56	85,64			

***AUC**: Area under the curve, **AVG**: Average area under the curve, **Conc**: Concentration in $\mu\text{g/ml}$, **Fin. Conc.**: Final concentration, **AVG. Conc**: Average concentration in $\mu\text{g/ml}$, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **P**: Pyrimethamine, **P**: PBS buffer solution, **1 – 18**: Sample number.

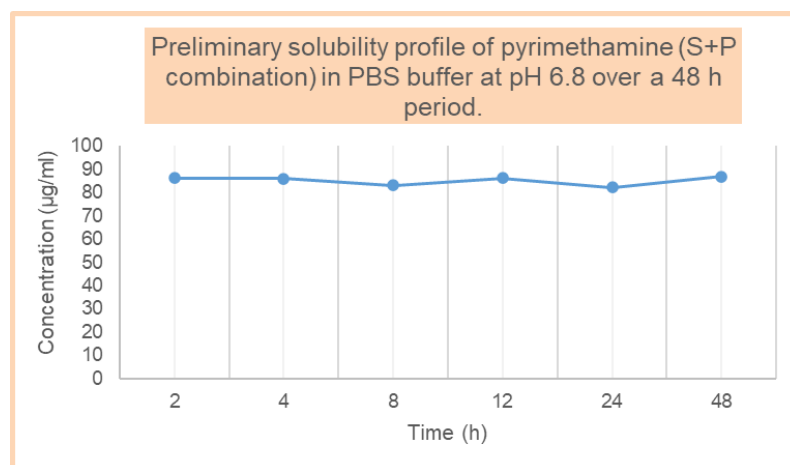


Figure 4.28: Preliminary solubility profile of pyrimethamine (in S + P combination) in PBS buffer solution.

As shown in Figure 4.28, pyrimethamine reached equilibrium within 2 hours of the study in the presence of sulfadoxine. Furthermore, pyrimethamine remained stable in the PBS buffer while in the presence of sulfadoxine over the entire study. The solubility of pyrimethamine, in the presence of sulfadoxine, increased by approximately 20 µg/ml in comparison with the single component.

4.3.2.5 Preliminary solubility in sodium phosphate (SBS) buffer solution at pH 6.8

Preliminary solubility results obtained for pyrimethamine (single component) in SBS buffer solution are presented in Table 4.26 and Figure 4.29.

Table 4.26: Preliminary solubility data of pyrimethamine in SBS buffer solution at pH 6.8

Preliminary - Pyrimethamine (Single) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	PS1	156830	151237	154034	7,35	80,89	79,60	1,44	1,81
	PS2	151155	152410	151783	7,26	79,86			
	PS3	147723	147839	147781	7,10	78,05			
4 h	PS4	142752	145246	143999	6,94	76,33	78,21	2,54	3,25
	PS5	154668	154315	154492	7,37	81,09			
	PS6	143335	148482	145909	7,02	77,20			
8 h	PS7	149664	148012	148838	7,14	78,53	76,19	5,12	6,72
	PS8	151225	151689	151457	7,25	79,72			
	PS9	136075	125440	130758	6,39	70,31			
12 h	PS10	146652	148697	147675	7,09	78,00	76,68	3,66	4,77
	PS11	141629	129703	135666	6,59	72,54			
	PS12	152247	149700	150974	7,23	79,50			
24 h	PS13	151164	148018	149591	7,17	78,87	79,66	2,78	3,49
	PS14	158610	157642	158126	7,52	82,75			
	PS15	145567	146977	146272	7,03	77,36			
48 h	PS16	144392	142891	143642	6,92	76,17	77,69	1,51	1,94
	PS17	146126	147976	147051	7,07	77,72			
	PS18	150331	150220	150276	7,20	79,18			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **P**: Pyrimethamine, **S**: SBS buffer solution, **1 – 18**: Sample number.

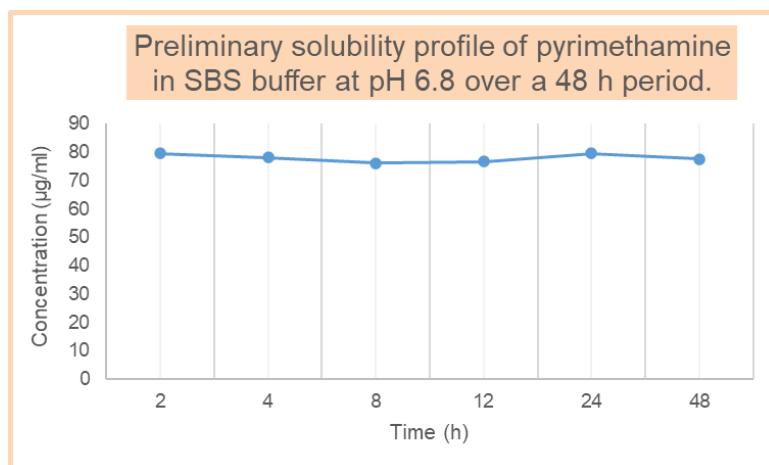


Figure 4.29: Preliminary solubility profile of pyrimethamine (single component) in SBS buffer solution.

It is evident from the data obtained that pyrimethamine entered into the solution rapidly and remained stable in solution during 48 h. It obtained a maximum solubility of approximately 79 µg/ml, and equilibrium was achieved rapidly.

Preliminary solubility results obtained for artesunate (A + P combination) in SBS buffer solution are presented in Table 4.27 and Figure 4.30.

Table 4.27: Preliminary solubility data of artesunate in the A+P combination in SBS buffer solution at pH 6.8

Preliminary - Pyrimethamine (A + P combination) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APS1	15845	9285	12565	1,51	151,12	137,53	11,78	8,57
	APS2	7657	7903	7780	1,31	131,36			
	APS3	7591	7369	7480	1,30	130,12			
4 h	APS4	97519	98872	98196	5,05	504,74	532,87	24,39	4,58
	APS5	108910	108467	108689	5,48	548,08			
	APS6	107321	108956	108139	5,46	545,80			
8 h	APS7	157619	156335	156977	7,47	747,49	745,97	23,05	3,09
	APS8	146640	155066	150853	7,22	722,20			
	APS9	157340	166656	161998	7,68	768,23			
12 h	APS10	235224	222044	228634	10,43	1043,41	945,43	87,14	9,22
	APS11	197999	197676	197838	9,16	916,23			
	APS12	187305	189195	188250	8,77	876,64			
24 h	APS13	375925	374369	375147	16,48	1648,46	1676,05	40,75	2,43
	APS14	378034	376315	377175	16,57	1656,83			
	APS15	393616	392704	393160	17,23	1722,85			
48 h	APS16	375383	374669	375026	16,48	1647,96	1708,23	96,96	5,68
	APS17	377512	376745	377129	16,57	1656,64			
	APS18	391710	441694	416702	18,20	1820,07			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **A:** Artesunate, **P:** Pyrimethamine, **S:** SBS buffer solution, **1 – 18:** Sample number.

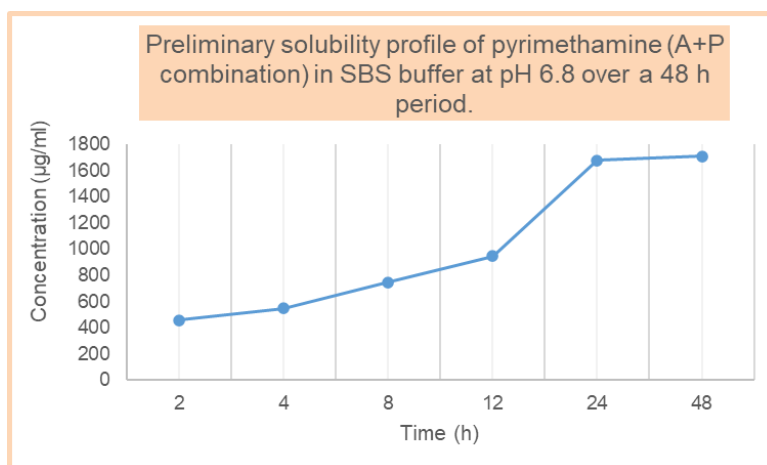


Figure 4.30: Preliminary solubility profile of artesunate (in A + P combination) in SBS buffer solution.

Based on the acquired data, pyrimethamine in the A + P combination attained an increase in solubility, achieving equilibrium at 24 hours with a maximum solubility of $\pm 1708 \mu\text{g/ml}$ at 48 h, which is a 21-fold increase compared to the single component profile. It is important to note that this enhancement cannot be solely attributed to the presence of artesunate; there is a possibility that it is also due to the degradation of artesunate. It can be postulated that this degradation may lead to the creation of a more acidic environment, which could be favourable for the solubility of pyrimethamine. The same trend can be observed for this combination in PBS, with the exception of equilibrium time, which was obtained earlier in PBS buffer.

The preliminary solubility results obtained for pyrimethamine (in S + P combination) in SBS buffer solution are presented in Table 4.28 and Figure 4.31.

Table 4.28: Preliminary solubility data of pyrimethamine in the S+P combination in SBS buffer at pH 6.8

Preliminary - Pyrimethamine (S + P combination) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPS1	226068	197882	211975	9,75	97,46	95,90	2,00	2,09
	SPS2	201960	217819	209890	9,66	96,60			
	SPS3	211363	194089	202726	9,36	93,64			
4 h	SPS4	197220	192118	194669	9,03	90,31	90,84	1,47	1,61
	SPS5	195609	190797	193203	8,97	89,71			
	SPS6	202317	197584	199951	9,25	92,50			
8 h	SPS7	203254	195388	199321	9,22	92,24	93,46	2,90	3,10
	SPS8	196776	197729	197253	9,14	91,38			
	SPS9	198324	222304	210314	9,68	96,78			
12 h	SPS10	184175	195675	189925	8,84	88,36	92,87	3,94	4,25
	SPS11	216184	199037	207611	9,57	95,66			
	SPS12	208148	201882	205015	9,46	94,59			
24 h	SPS13	219106	189520	204313	9,43	94,30	91,52	2,45	2,68
	SPS14	195975	194804	195390	9,06	90,61			
	SPS15	193287	192816	193052	8,96	89,65			
48 h	SPS16	217242	206962	212102	9,75	97,51	92,56	4,30	4,64
	SPS17	194539	195295	194917	9,04	90,42			
	SPS18	194553	192115	193334	8,98	89,76			

***AUC**: Area under the curve, **AVG**: Average area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **P**: Pyrimethamine, **S**: SBS buffer solution, **1 – 18**: Sample number.

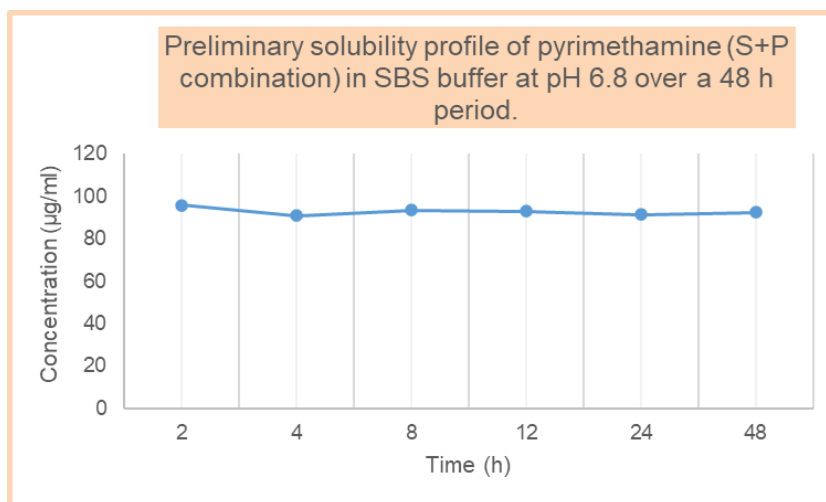


Figure 4.31: Preliminary solubility profile of pyrimethamine (in S + P combination) in SBS buffer solution.

The data presented in Figure 4.31 indicates a slight increase in the solubility of pyrimethamine when combined with sulfadoxine in comparison to the single component. Pyrimethamine demonstrated rapid solubility and maintained stability throughout the 48 h study period. Specifically, in the S + P combination, the maximum solubility of pyrimethamine was approximately 95 µg/ml at 2 h.

4.3.2.6 Preliminary solubility in water

Preliminary solubility results obtained for pyrimethamine (single component) in ultra-purified water are presented in Table 4.29 and Figure 4.32.

Table 4.29: Preliminary solubility data of pyrimethamine in water

Preliminary - Pyrimethamine (Single) in water								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	PW1	355441	335384	345413	15,36	17,86	5,57	31,19
	PW2	562697	567428	565063	24,24			
	PW3	308919	313720	311320	13,98			
4 h	PW4	250047	260086	255067	11,70	13,40	1,47	11,00
	PW5	312398	316748	314573	14,11			
	PW6	320234	322240	321237	14,38			
8 h	PW7	291016	298680	294848	13,31	14,53	1,31	9,01
	PW8	355687	362640	359164	15,91			
	PW9	321081	320247	320664	14,36			
12 h	PW10	273484	270392	271938	12,39	11,68	0,63	5,37
	PW11	249247	248378	248813	11,45			
	PW12	240158	244848	242503	11,20			
24 h	PW13	188992	191474	190233	9,08	10,23	1,06	10,35
	PW14	223987	224382	224185	10,45			
	PW15	239800	243701	241751	11,16			
48 h	PW16	248462	253776	251119	11,54	10,86	0,69	6,32
	PW17	234607	233924	234266	10,86			
	PW18	212248	222142	217195	10,17			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering: P:** Pyrimethamine, **W:** Water, **1 – 18:** Sample number.

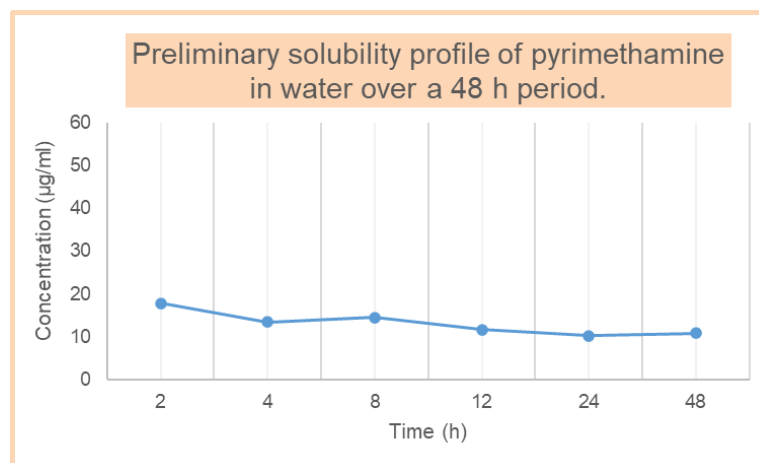


Figure 4.32: Preliminary solubility profile of pyrimethamine (single component) in water.

It is clear from the results above that pyrimethamine is poorly soluble in water, and it reached a maximum solubility of approximately 17 µg/ml within 2 h. Pyrimethamine remained stable in solution and maintained equilibrium throughout the 48 h preliminary study. The %RSDs were high during the experiment. However, the variations can be attributed to the fact that the concentrations obtained were very low.

Preliminary solubility results obtained for pyrimethamine (A + P combination) in ultra-purified water are presented in Table 4.30 and Figure 4.33.

Table 4.30: Preliminary solubility data of pyrimethamine in the A+P combination in water

Preliminary - Pyrimethamine (A + P combination) in water									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	APW1	276377	272276	274327	12,48	1248,25	1269,63	0,40	0,03
	APW2	289594	292384	290989	13,16	1315,64			
	APW3	274476	272570	273523	12,45	1245,00			
4 h	APW4	271067	272020	271544	12,37	1236,99	1375,21	1,30	0,09
	APW5	336828	333908	335368	14,95	1495,12			
	APW6	310376	310116	310246	13,94	1393,52			
8 h	APW7	399538	400152	399845	17,56	1755,90	1820,97	1,70	0,09
	APW8	382505	386162	384334	16,93	1693,16			
	APW9	463706	463534	463620	20,14	2013,83			
12 h	APW10	424492	423700	424096	18,54	1853,98	1918,92	0,56	0,03
	APW11	441628	455580	448604	19,53	1953,10			
	APW12	446615	448906	447761	19,50	1949,69			
24 h	APW13	661777	661845	661811	28,15	2815,41	2801,14	0,89	0,03
	APW14	677823	678640	678232	28,82	2881,82			
	APW15	638247	631375	634811	27,06	2706,21			
48 h	APW16	877807	877726	877767	36,89	3688,83	3901,73	2,18	0,06
	APW17	928907	927056	927982	38,92	3891,92			
	APW18	985042	985905	985474	41,24	4124,45			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **A**: Artesunate, **P**: Pyrimethamine, **W**: water, **1 – 18**: Sample number.

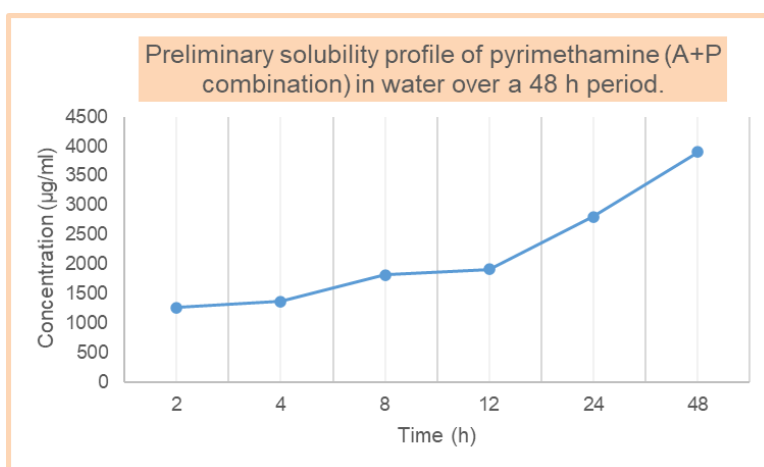


Figure 4.33: Preliminary solubility profile of pyrimethamine (in A + P combination) in water.

Based on the acquired data, pyrimethamine in the A + P combination attained an increase in solubility with a maximum solubility of $\pm 3901 \mu\text{g/ml}$ at 48 h, which is a 229-fold increase compared to the single component profile. It is important to note that this enhancement cannot be solely attributed to the presence of artesunate; there is a possibility that it is also due to the degradation of artesunate. In the study done by [Al Haydar, \(2011\)](#), it was confirmed that artesunate degrades when the succinate ester comes into contact with water. This hydrolysis happens faster when more hydronium or hydroxide ions are present. This degradation leads to the creation of a more acidic environment, which is favourable for the solubility of pyrimethamine.

The preliminary solubility results obtained for pyrimethamine (in S + P combination) in water are presented in Table 4.31 and Figure 4.34.

Table 4.31: Preliminary solubility data of pyrimethamine in the S+P combination in water

Preliminary - Pyrimethamine (S + P combination) in water									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. ($\mu\text{g/ml}$)	AVG. Conc. ($\mu\text{g/ml}$)	SD	%RSD
2 h	SPW1	304411	334487	319449	14,31	143,07	148,44	5,38	3,62
	SPW2	328337	336980	332659	14,84	148,42			
	SPW3	346790	345326	346058	15,38	153,84			
4 h	SPW4	303852	324016	313934	14,08	140,84	150,52	8,51	5,65
	SPW5	347178	345167	346173	15,39	153,88			
	SPW6	355841	351101	353471	15,68	156,83			
8 h	SPW7	304697	334856	319777	14,32	143,21	150,96	6,96	4,61
	SPW8	325753	362377	344065	15,30	153,03			
	SPW9	350984	355069	353027	15,67	156,65			
12 h	SPW10	364968	358417	361693	16,02	160,16	171,97	15,34	8,92
	SPW11	378490	375955	377223	16,64	166,44			
	SPW12	418262	449221	433742	18,93	189,30			
24 h	SPW13	310381	334369	322375	14,43	144,26	153,67	8,35	5,43
	SPW14	345201	378371	361786	16,02	160,20			
	SPW15	369418	336082	352750	15,65	156,54			
48 h	SPW16	384913	387129	386021	17,00	170,00	163,14	8,62	5,29
	SPW17	360319	329915	345117	15,35	153,46			
	SPW18	349116	402927	376022	16,60	165,95			

***AUC**: Area under the curve, **AVG**: Average area under the curve, **Conc**: Concentration in $\mu\text{g/ml}$, **Fin. Conc.**: Final concentration, **AVG. Conc**: Average concentration in $\mu\text{g/ml}$, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **P**: Pyrimethamine, **W**: Water, **1 – 18**: Sample number.

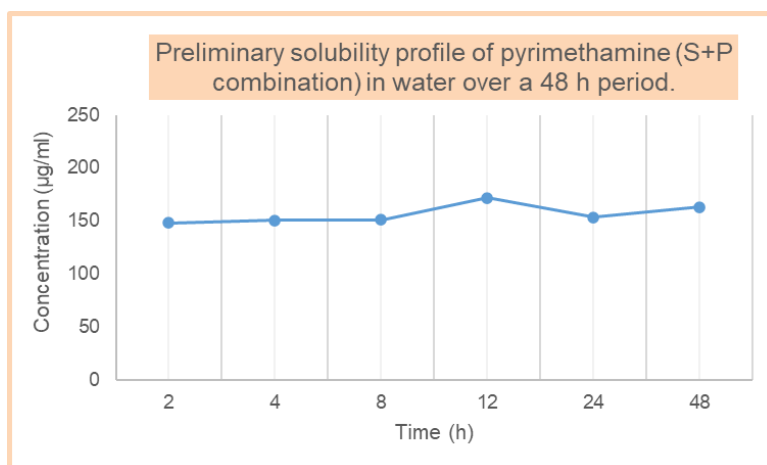


Figure 4.34: Preliminary solubility profile of pyrimethamine (in S + P combination) in water.

It is clear from Figure 34 that the solubility of pyrimethamine in the presence of sulfadoxine increased 10-fold. Pyrimethamine rapidly solubilised and maintained stability during the 48 h study. The maximum solubility of pyrimethamine in S + P combination was $\pm 171 \mu\text{g/ml}$ at 12 h.

4.3.2.7 Pivot solubility of pyrimethamine, and pyrimethamine in the A + P combination

In Table 4.32 and Figure 4.35 below are compilations of the pivot solubility studies performed for pyrimethamine as a single component and in combination with artesunate in the various solubility media ranging from pH 1.2 – 6.8. Please note that due to artesunate's instability in the various media, it was decided that withdrawal for the pivot solubility studies would be performed at 2 hours.

Table 4.32: Pivot solubility of pyrimethamine in single component and A + P combination in various media.

	Average Concentration ($\mu\text{g/ml}$)					
	Pyri (single)	SD	%RSD	Pyri (A+P Combo)	SD	%RSD
Solvent						
0.1 N HCl	2917,08	0,75	0,03	2823,77	2,05	0,08
NaCl	2185,77	0,63	0,03	2203,20	0,21	0,01
ACE	4685,80	1,36	0,03	2945,43	2,06	0,07
PBS	66,60	0,16	0,24	445,22	0,07	0,02
SBS	80,75	0,34	0,42	699,65	0,29	0,04
Water	21,57	1,24	5,73	1006,82	0,32	0,03

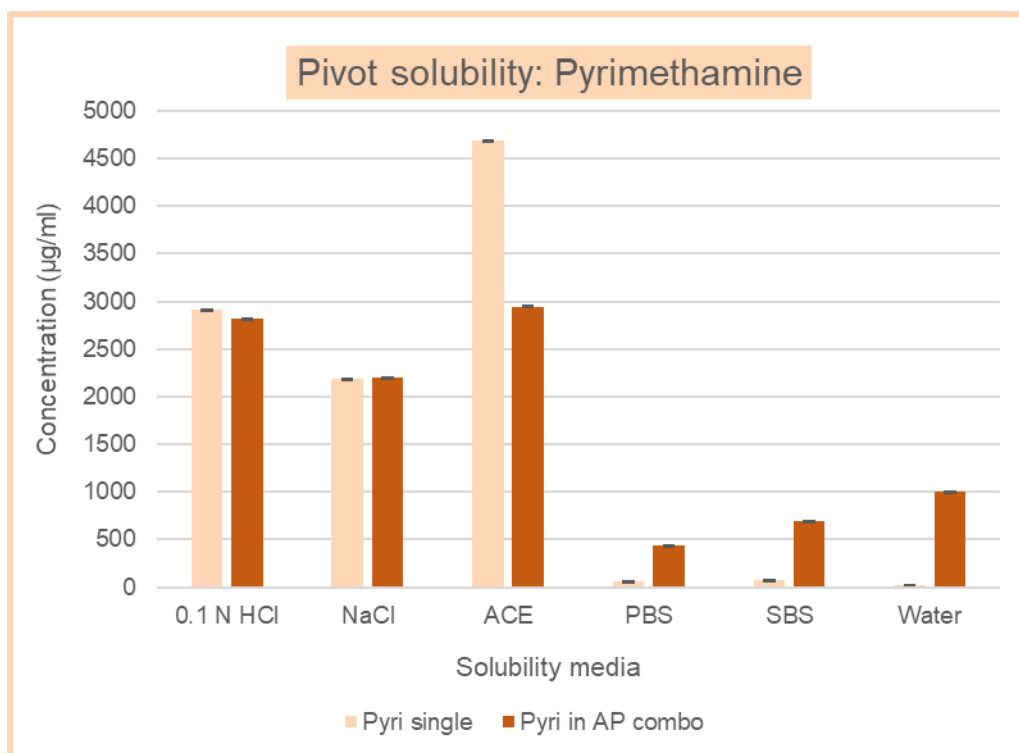


Figure 4.35: Pivot solubility of pyrimethamine in single component and A + P combination in various media.

Table 4.32 and Figure 4.35 indicate an enhancement in the solubility of pyrimethamine when combined with artesunate, specifically in PBS, SBS, and in water, compared to its solubility as a single component. On the contrary, lower solubility profiles were observed for pyrimethamine in combination with artesunate in a 0.1 N HCl solution and an ACE buffer solution, while the solubility remained relatively consistent in the NaCl buffer solution.

Remarkably, the most substantial increase in pyrimethamine's solubility occurred when combined with artesunate in water, surpassing a 46-fold increase compared to its solubility as a single component. This was then followed by SBS and PBS buffer solutions, where the increase was 8 times and 6 times higher, respectively.

4.3.2.8 Pivot solubility for pyrimethamine and pyrimethamine in the S + P combination

Table 4.33 and Figure 4.36 below are compilations of the pivot solubility studies performed for pyrimethamine as a single component and in combination with sulfadoxine in the various solubility media ranging from pH 1.2 – 6.8. The pivot solubility study for this combination was performed at 2 h since equilibrium for pyrimethamine was achieved rapidly in all media.

Table 4.33: Pivot solubility of pyrimethamine in single component and S + P combination in various media.

Solvent	Average Concentration ($\mu\text{g/ml}$)					
	Pyri (single)	SD	%RSD	Pyri (S+P Combo)	SD	%RSD
0.1 N HCl	2917,08	0,75	0,03	2806,95	0,73	0,03
NaCl	2185,77	0,63	0,03	2165,38	0,82	0,04
ACE	4685,80	1,36	0,03	4564,79	2,75	0,06
PBS	66,60	0,16	0,24	92,83	0,20	0,22
SBS	80,75	0,34	0,42	112,27	0,11	0,10
Water	21,57	1,24	5,73	175,72	0,61	0,35

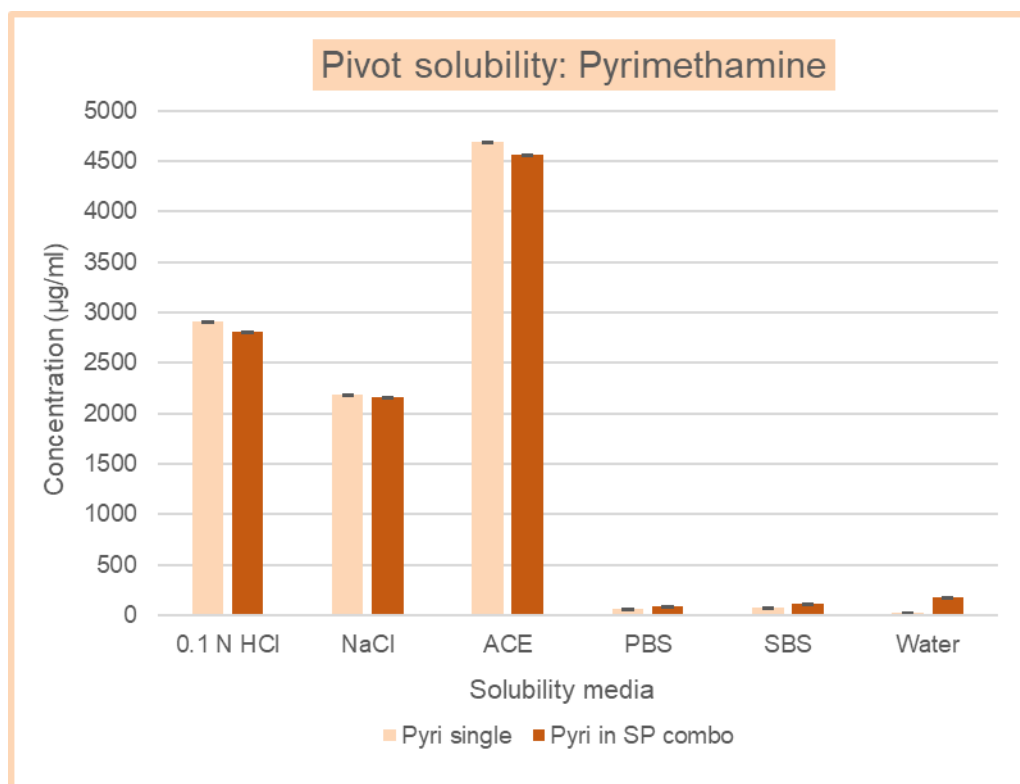


Figure 4.36: Pivot solubility of pyrimethamine in single component and S + P combination in various media.

Upon examination of the data in Figure 4.36 and Table 4.33, it was evident that pyrimethamine produced the same trend with regards to the solubility profile across the pH range of 1.2 to 6.8, with the inclusion of water, as with the A + P combination. The most noteworthy differences

were in combination, whereby pyrimethamine produced higher solubility values in combination with artesunate in higher pH values and water compared to its combination with sulfadoxine. Similar to the combination with artesunate, the most pronounced increase was in water, whereby pyrimethamine's solubility increased 8-fold when in combination with sulfadoxine. This correlates with the study performed by [Badenhorst \(2017\)](#).

4.3.3 Sulfadoxine

This section delves into the discussion of sulfadoxine both as single component and in combination with pyrimethamine (500 mg S + 25 mg P). Preliminary solubility studies were conducted in water and various buffers/solvents ranging from pH 1.2 – 6.8. In all preliminary experiments, the pH of the solvents was measured before initiating the experiment. Subsequently, the pH of the solution (solute + solvent) was taken afterward at the various time intervals. Although there is a noticeable degree of variability in some data sets of sulfadoxine with the reported high % RSD, it is crucial to recognise the intrinsic complexity of the studied phenomena. To completely understand the contributing elements to this variability, further investigation and experimentation could be necessary. This admission creates opportunities for additional study to improve and deepen our comprehension of the observed differences.

4.3.3.1 Preliminary solubility in 0.1 N HCl solution at pH 1.2

Preliminary solubility results obtained for sulfadoxine (single component) in 0.1 N HCl solution are presented in Table 4.34 and Figure 4.37.

Table 4.34: Preliminary solubility data of sulfadoxine in 0.1 N HCl at pH 1.2

Preliminary - Sulfadoxine (Single) in HCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SH1	3910825	3904759	3907792	102,06	1020,63	1022,13	13,73	1,34
	SH2	3873631	3857242	3865437	100,92	1009,21			
	SH3	3967932	3965761	3966847	103,65	1036,55			
4 h	SH4	3963849	3958837	3961343	103,51	1035,07	1036,45	12,82	1,24
	SH5	3922988	3920450	3921719	102,44	1024,38			
	SH6	4015940	4016874	4016407	104,99	1049,91			
8 h	SH7	3982094	3986464	3984279	104,12	1041,25	1023,22	20,36	1,99
	SH8	3831717	3839267	3835492	100,11	1001,14			
	SH9	3930835	3934108	3932472	102,73	1027,28			
12 h	SH10	3842051	3840369	3841210	100,27	1002,68	1004,01	37,90	3,78
	SH11	3707119	3709015	3708067	96,68	966,79			
	SH12	3988977	3989332	3989155	104,26	1042,56			
24 h	SH13	3994411	3991072	3992742	104,35	1043,53	1055,63	13,79	1,31
	SH14	4026876	4026748	4026812	105,27	1052,71			
	SH15	4093488	4093218	4093353	107,06	1070,65			
48 h	SH16	4118868	4116223	4117546	107,72	1077,17	1088,59	24,65	2,26
	SH17	4267637	4262026	4264832	111,69	1116,87			
	SH18	4298773	3895807	4097290	107,17	1071,71			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **H**: HCl solution, **1 – 18**: Sample number.

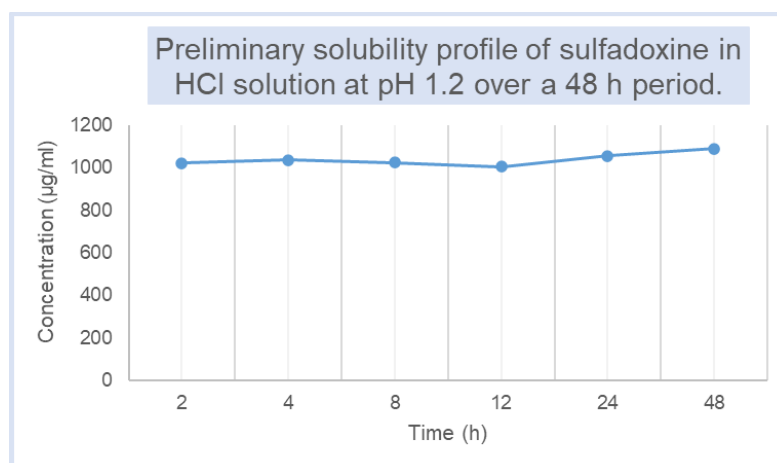


Figure 4.37: Preliminary solubility profile of sulfadoxine (single component) in 0.1 N HCl solution.

From Figure 4.37 and Table 4.34, it is evident that sulfadoxine attained its maximal solubility of approximately 1088 µg/ml within 48 h. However, from 2 h to 48 h there was little variation in the concentration at each interval. Therefore, it can be said that sulfadoxine went quickly into solution and remained in equilibrium in 0.1 N HCl solution over the 48 h period.

The preliminary solubility results obtained for sulfadoxine (in S + P combination) in 0.1 N HCl solution are presented in Table 4.35 and Figure 4.38.

Table 4.35: Preliminary solubility data of sulfadoxine in the S+P combination in 0.1 N HCl at pH 1.2

Preliminary - Sulfadoxine (S+P Combination) in HCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPH1	374210	340012	357111	6,35	635,08	724,35	110,32	15,23
	SPH2	436423	435540	435982	8,48	847,68			
	SPH3	371216	383985	377601	6,90	690,31			
4 h	SPH4	313353	308772	311063	5,11	510,95	545,29	30,16	5,53
	SPH5	328575	328076	328326	5,57	557,48			
	SPH6	317755	346293	332024	5,67	567,45			
8 h	SPH7	383911	348860	366386	6,60	660,08	668,33	92,61	13,86
	SPH8	351323	322128	336726	5,80	580,12			
	SPH9	409319	401148	405234	7,65	764,79			
12 h	SPH10	408204	409943	409074	7,75	775,15	731,22	62,13	8,50
	SPH11	386455	366505	376480	6,87	687,29			
	SPH12	No data							
24 h	SPH13	356097	327459	341778	5,94	593,74	639,54	44,00	6,88
	SPH14	377016	343361	360189	6,43	643,37			
	SPH15	357408	391258	374333	6,81	681,50			
48 h	SPH16	362006	362645	362326	6,49	649,13	676,34	103,97	15,37
	SPH17	397683	432387	415035	7,91	791,22			
	SPH18	340856	338942	339899	5,89	588,68			

***AUC**: Area under the curve, **AVG**: Average area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **P**: Pyrimethamine, **H**: HCl solution, **1 – 18**: Sample number.

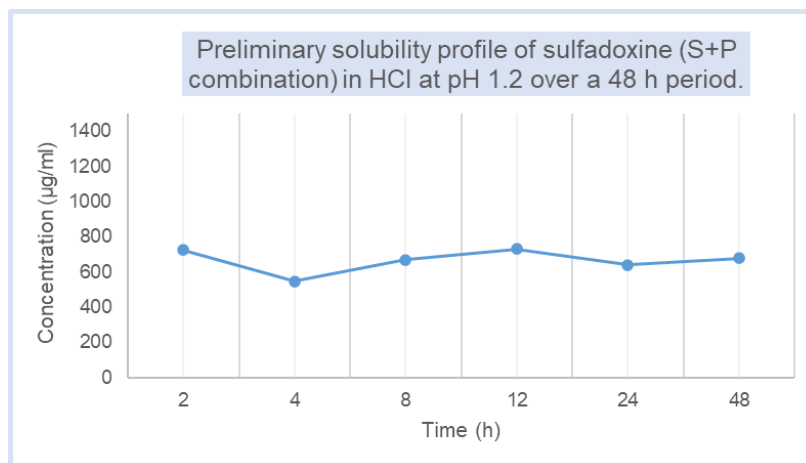


Figure 4.38: Preliminary solubility profile of artesunate (in S + P combination) in 0.1 N HCl solution.

Sulfadoxine solubilised immediately and remained stable in solution, where maximal solubility was attained within 12 h with a concentration of 731 µg/ml. The solubility of sulfadoxine in the presence of pyrimethamine appears to be lower (± 350 µg/ml less) than in comparison to the single component. This too correlates with a study performed by [Badenhorst \(2017\)](#).

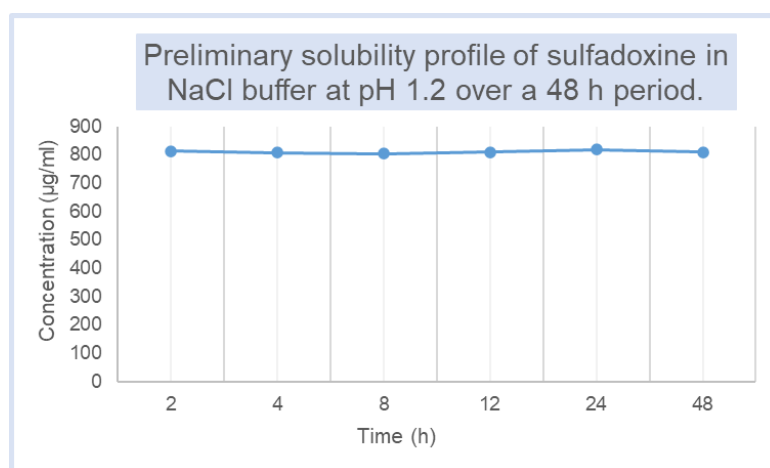
4.3.3.2 Preliminary solubility in NaCl buffer solution at pH 1.2

Preliminary solubility results obtained for sulfadoxine (single component) in NaCl buffer solution are presented in Table 4.36 and Figure 4.39.

Table 4.36: Preliminary solubility data of sulfadoxine in NaCl buffer solution at pH 1.2

Preliminary - Sulfadoxine (Single) in NaCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SN1	3132748	3135759	3134254	80,15	801,49	814,24	12,59	1,55
	SN2	3185000	3183540	3184270	81,46	814,57			
	SN3	3232424	3228668	3230546	82,67	826,67			
4 h	SN4	3150889	3157970	3154430	80,68	806,77	808,39	2,71	0,34
	SN5	3167330	3177872	3172601	81,15	811,52			
	SN6	3150078	3159667	3154873	80,69	806,88			
8 h	SN7	3163924	3166763	3165344	80,96	809,62	804,29	8,75	1,09
	SN8	3110419	3102321	3106370	79,42	794,20			
	SN9	3163097	3163318	3163208	80,91	809,06			
12 h	SN10	3137822	3125382	3131602	80,08	800,80	810,50	9,01	1,11
	SN11	3199523	3199794	3199659	81,86	818,59			
	SN12	3176974	3172638	3174806	81,21	812,10			
24 h	SN13	3176731	3178609	3177670	81,28	812,84	819,06	8,57	1,05
	SN14	3187603	3188023	3187813	81,55	815,50			
	SN15	3244204	3233481	3238843	82,88	828,84			
48 h	SN16	2992761	3292195	3142478	80,36	803,64	809,95	15,30	1,89
	SN17	3008861	3239186	3124024	79,88	798,81			
	SN18	3233951	3232741	3233346	82,74	827,40			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **N**: NaCl buffer solution, **1 – 18**: Sample number.

**Figure 4.39:** Preliminary solubility profile of sulfadoxine (single component) in NaCl buffer solution.

From Figure 4.39, it can be seen that sulfadoxine dissolved rapidly and stayed in equilibrium for the whole 48 h period. The maximum solubility was approximately 819 µg/ml within 24 h however, the variance in concentration between each time interval (from 2 h -48 h) was very low.

Preliminary solubility results obtained for sulfadoxine (S + P combination) in NaCl buffer solution are presented in Table 4.37 and Figure 4.40.

Table 4.37: Preliminary solubility data of sulfadoxine in the S+P combination in NaCl buffer solution at pH 1.2

Preliminary - Sulfadoxine (S+P combination) in NaCl									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPN1	402432	403913	403173	8,72	872,50	742,39	112,70	15,18
	SPN2	346981	311975	329478	6,80	679,77			
	SPN3	317099	338137	327618	6,75	674,90			
4 h	SPN4	315197	305310	310254	6,29	629,49	624,64	7,25	1,16
	SPN5	308573	310891	309732	6,28	628,13			
	SPN6	291260	319170	305215	6,16	616,31			
8 h	SPN7	325825	356379	341102	7,10	710,17	705,46	6,66	0,94
	SPN8	No data							
	SPN9	338064	336937	337501	7,01	700,75			
12 h	SPN10	318341	315932	317137	6,47	647,49	649,09	2,44	0,38
	SPN11	316094	318472	317283	6,48	647,88			
	SPN12	319294	318354	318824	6,52	651,91			
24 h	SPN13	377813	375933	376873	8,04	803,72	831,19	38,85	4,67
	SPN14	No data							
	SPN15	397397	398368	397883	8,59	858,66			
48 h	SPN16	365502	373478	369490	7,84	784,41	745,63	34,17	4,58
	SPN17	344353	345301	344827	7,20	719,91			
	SPN18	336261	363080	349671	7,33	732,58			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **S:** Sulfadoxine, **P:** Pyrimethamine, **N:** NaCl buffer solution, **1 – 18:** Sample number.

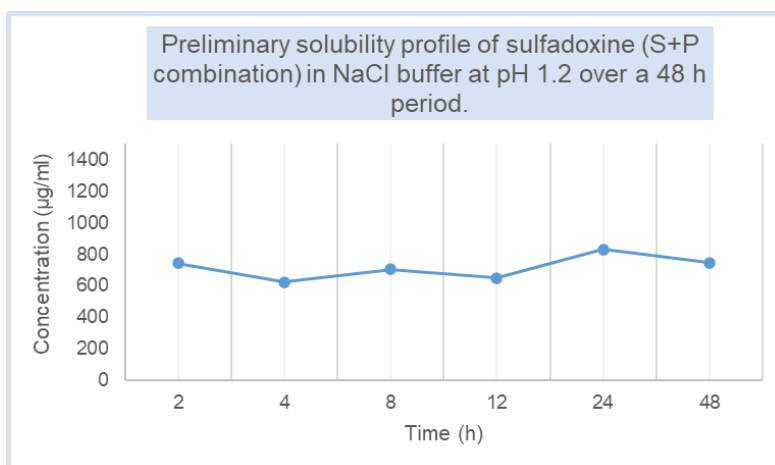


Figure 4.40: Preliminary solubility profile of sulfadoxine (in S + P combination) in NaCl buffer solution.

From Figure 4.40, it can be seen that sulfadoxine exhibited a rapid solubility process, maintaining equilibrium throughout the experiment. It had a maximum solubility of $\pm 831 \mu\text{g/ml}$, as seen in Table 4.34 within 24h. The solubility appeared to be slightly lower than in comparison to the single component.

4.3.3.3 Preliminary solubility in acetate (ACE) buffer solution at pH 4.5

Preliminary solubility results obtained for sulfadoxine (single component) in ACE buffer solution are presented in Table 4.38 and Figure 4.41.

Table 4.38: Preliminary solubility data of sulfadoxine in ACE buffer solution at pH 4.5

Preliminary - Sulfadoxine (Single) in ACE								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SA1	7496775	7496467	7496621	192,04	169,58	36,10	21,29
	SA2	5130129	4975899	5053014	127,93			
	SA3	7377416	7367104	7372260	188,78			
4 h	SA4	8035524	8019694	8027609	205,97	183,86	57,54	31,30
	SA5	4694962	4695484	4695223	118,55			
	SA6	8832178	8832440	8832309	227,08			
8 h	SA7	8118810	8084213	8101512	207,91	193,49	15,60	8,06
	SA8	7650393	7618046	7634220	195,65			
	SA9	6926127	6915034	6920581	176,93			
12 h	SA10	7121447	7099683	7110565	181,91	185,16	9,32	5,03
	SA11	6972230	6943517	6957874	177,90			
	SA12	7285774	7984197	7634986	195,67			
24 h	SA13	7945308	8755451	8350380	214,43	195,92	30,46	15,55
	SA14	8279208	8278827	8279018	212,56			
	SA15	6313328	6295032	6304180	160,76			
48 h	SA16	8770380	7954300	8362340	214,75	209,63	8,58	4,09
	SA17	7810426	7768732	7789579	199,72			
	SA18	8755541	7944780	8350161	214,43			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering: S:** Sulfadoxine, **A:** ACE buffer solution, **1 – 18:** Sample number.

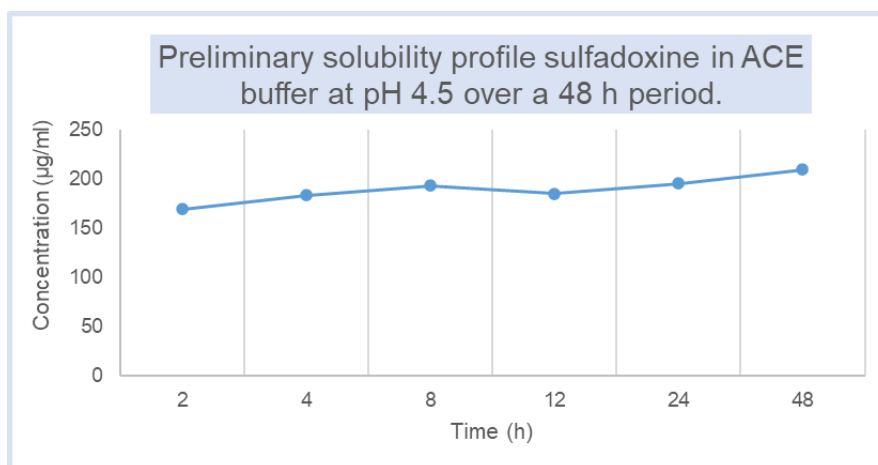


Figure 4.41: Preliminary solubility profile of sulfadoxine (single component) in ACE buffer solution.

From the Figure 4.41, it was evident that sulfadoxine solubilise rapidly (at 2 h), maintaining equilibrium for 48 h with minimum variation in the average concentration obtained between each time interval. Therefore, it can be inferred that sulfadoxine is stable in ACE buffer at pH 4.5. From the data in Table 4.35, the maximum solubility was $\pm 209 \mu\text{g/ml}$ at 48 h.

Preliminary solubility results obtained for sulfadoxine (S + P combination) in ACE buffer solution are presented in Table 4.39 and Figure 4.42.

Table 4.39: Preliminary solubility data of sulfadoxine in the S+P combination in ACE buffer solution at pH 4.5

Preliminary - Sulfadoxine (S + P Combination) in ACE								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. ($\mu\text{g/ml}$)	AVG. Conc. ($\mu\text{g/ml}$)	SD	%RSD
2 h	SP A1	9847808	9892349	9870079	254,30	253,03	2,31	0,91
	SP A2	9717786	9722408	9720097	250,37			
	SP A3	9795132	9954488	9874810	254,42			
4 h	SP A4	10393719	10154807	10274263	264,90	249,43	15,99	6,41
	SP A5	9755298	9688213	9721756	250,41			
	SP A6	9059006	9054763	9056885	232,97			
8 h	SP A7	9959809	9948096	9953953	256,50	258,76	1,98	0,76
	SP A8	10096229	10090543	10093386	260,16			
	SP A9	10116156	10029731	10072944	259,62			
12 h	SP A10	10649450	10672760	10661105	275,05	279,17	3,66	1,31
	SP A11	10867143	10863294	10865219	280,41			
	SP A12	10915818	10940826	10928322	282,06			
24 h	SP A13	10876002	10832560	10854281	280,12	278,08	6,49	2,33
	SP A14	10494995	10504005	10499500	270,81			
	SP A15	11019217	10931553	10975385	283,30			
48 h	SP A16	10621340	10628489	10624915	274,10	281,26	8,28	2,94
	SP A17	11143467	11343420	11243444	290,33			
	SP A18	10837607	10811400	10824504	279,34			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in $\mu\text{g/ml}$, **AVG. Conc:** Average concentration in $\mu\text{g/ml}$, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **S:** Sulfadoxine, **P:** Pyrimethamine, **A:** ACE buffer solution, **1 – 18:** Sample number.

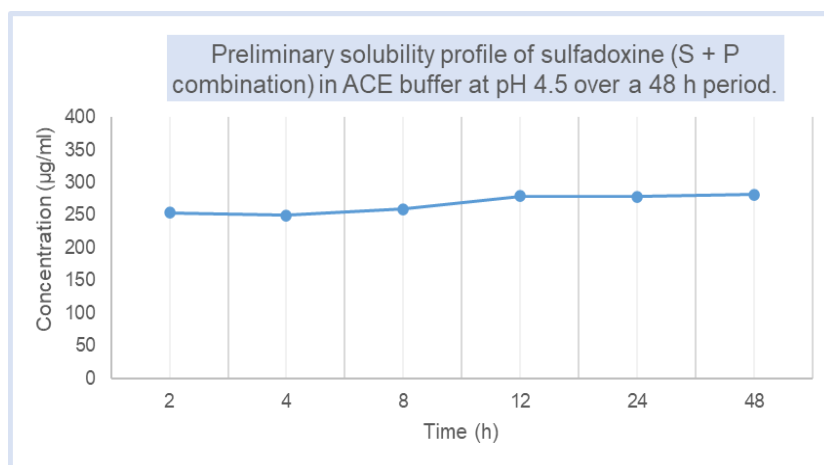


Figure 4.42: Preliminary solubility profile of sulfadoxine (in S + P combination) in ACE buffer solution.

The experimental results clearly indicated that sulfadoxine rapidly solubilised within 2 h and maintained equilibrium throughout the 48 h study. This suggested the stability of sulfadoxine in the presence of pyrimethamine within the ACE buffer at pH 4.5. From the data in Table 4.36, a maximum solubility of approximately 281 µg/ml was attained within 48 h, which was a slight improvement compared to the single component.

4.3.3.4 Preliminary solubility in potassium phosphate (PBS) buffer solution at pH 6.8

Preliminary solubility results obtained for sulfadoxine (single component) in PBS buffer solution are presented in Table 4.40 and Figure 4.43.

Table 4.40: Preliminary solubility data of sulfadoxine in PBS buffer solution at pH 6.8

Preliminary - Sulfadoxine (Single) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SP1	5434204	5424059	5429132	140,38	1403,80	1410,34	7,86	0,56
	SP2	5444830	5446622	5445726	140,82	1408,16			
	SP3	5488565	5485683	5487124	141,91	1419,06			
4 h	SP4	5644691	5656963	5650827	146,21	1462,14	1567,52	161,02	10,27
	SP5	6767070	6743891	6755481	175,29	1752,87			
	SP6	5747966	5746775	5747371	148,76	1487,55			
8 h	SP7	5697663	5697914	5697789	147,45	1474,50	1463,95	14,90	1,02
	SP8	5605532	5580343	5592938	144,69	1446,91			
	SP9	5684487	5680224	5682356	147,04	1470,44			
12 h	SP10	5733304	5725920	5729612	148,29	1482,88	1468,50	20,33	1,38
	SP11	5624012	5616752	5620382	145,41	1454,13			
	SP12	No data							
24 h	SP13	5510039	5503948	5506994	142,43	1424,29	1430,21	30,35	2,12
	SP14	5642572	5666208	5654390	146,31	1463,08			
	SP15	5422696	5431457	5427077	140,33	1403,25			
48 h	SP16	5622737	5620810	5621774	145,45	1454,50	1457,18	16,10	1,10
	SP17	5688567	5706668	5697618	147,45	1474,46			
	SP18	5572744	5580375	5576560	144,26	1442,60			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering: S**: Sulfadoxine, **P**: PBS buffer solution, **1 – 18**: Sample number.

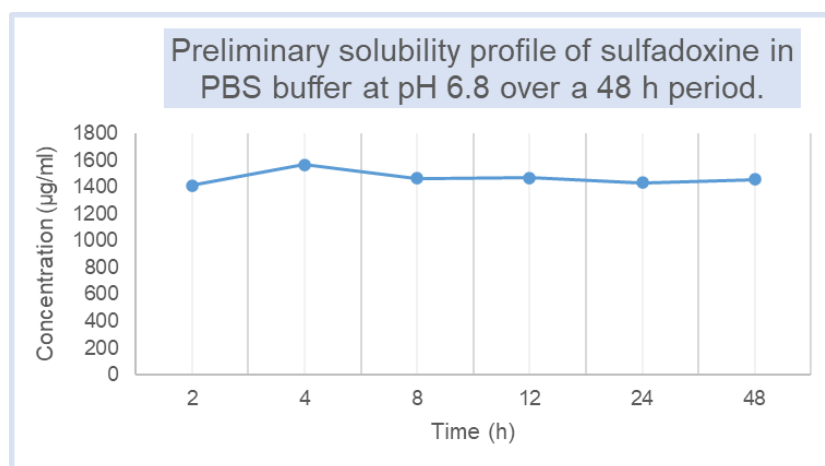


Figure 4.43: Preliminary solubility profile of sulfadoxine (single component) in PBS buffer solution.

It is clear that sulfadoxine rapidly reached its maximum solubility and remained stable in solution during the 48 hours. Its maximal solubility was $\pm 1567 \mu\text{g/ml}$ at 4 h, however early in the experiment equilibrium was attained and maintained.

Preliminary solubility results obtained for sulfadoxine (S + P combination) in PBS buffer solution are presented in Table 4.41 and Figure 4.44.

Table 4.41: Preliminary solubility data of sulfadoxine in the S + P combination in PBS buffer solution at pH 6.8

Preliminary - Sulfadoxine (S +P combination) in PBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. ($\mu\text{g/ml}$)	AVG. Conc. ($\mu\text{g/ml}$)	SD	%RSD
2 h	SPP1	4064123	4033687	4048905	104,05	1040,54	1228,57	180,80	14,72
	SPP2	4832215	4811814	4822015	124,40	1244,01			
	SPP3	5402096	5436110	5419103	140,12	1401,16			
4 h	SPP4	5176361	5033791	5105076	131,85	1318,51	1329,72	18,18	1,37
	SPP5	5109728	5111406	5110567	132,00	1319,95			
	SPP6	5118945	5335824	5227385	135,07	1350,70			
8 h	SPP7	5451663	5940147	5695905	147,40	1474,01	1433,77	49,22	3,43
	SPP8	5328231	5340811	5334521	137,89	1378,90			
	SPP9	5627897	5569477	5598687	144,84	1448,42			
12 h	SPP10	5194453	5180090	5187272	134,01	1340,14	1446,88	96,98	6,70
	SPP11	5881985	5932088	5907037	152,96	1529,57			
	SPP12	5491219	5877187	5684203	147,09	1470,93			
24 h	SPP13	5815448	5832042	5823745	150,77	1507,65	1448,18	51,97	3,59
	SPP14	5598179	5318220	5458200	141,14	1411,45			
	SPP15	5568211	5454654	5511433	142,55	1425,46			
48 h	SPP16	6018124	6013071	6015598	155,81	1558,14	1475,60	73,37	4,97
	SPP17	5506366	5458533	5482450	141,78	1417,83			
	SPP18	5375002	5840583	5607793	145,08	1450,82			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in $\mu\text{g/ml}$, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in $\mu\text{g/ml}$, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **P**: Pyrimethamine, **P**: PBS buffer solution, **1 – 18**: Sample number.

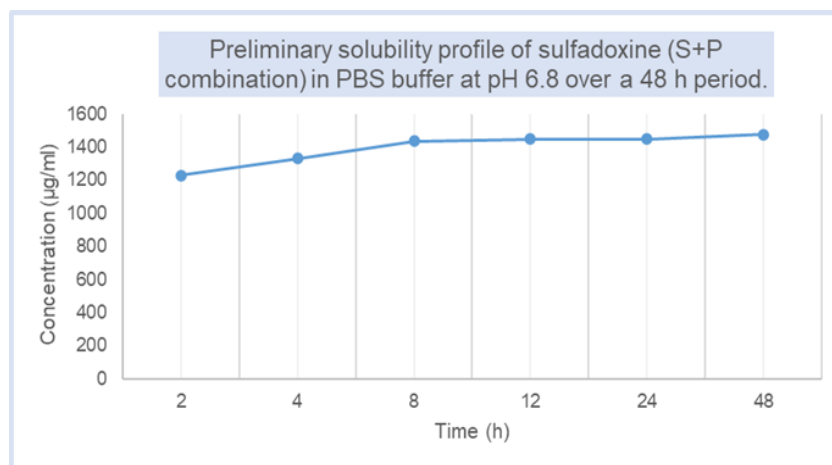


Figure 4.44: Preliminary solubility profile of sulfadoxine (in S + P combination) in PBS buffer solution.

As shown in Figure 4.44, sulfadoxine gradually solubilise for the initial 8 h, then reached equilibrium in the presence of pyrimethamine. Furthermore, sulfadoxine remained stable in the PBS buffer, whilst in the presence of pyrimethamine over the entire study. The maximum solubility of sulfadoxine, in the presence of pyrimethamine, was 1475 µg/ml as seen in Table 4.38. A very slight decrease in solubility (with approximately 90 µg/ml) for the combination was seen in comparison to the single component.

4.3.3.5 Preliminary solubility in sodium phosphate (SBS) buffer solution at pH 6.8

Preliminary solubility results obtained for sulfadoxine (single component) in SBS buffer solution are presented in Table 4.42 and Figure 4.45.

Table 4.42: Preliminary solubility data of sulfadoxine in SBS buffer solution at pH 6.8

Preliminary - Sulfadoxine (Single) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SS1	3987578	4040713	4014146	103,29	1136,21	1182,1	137,23	11,61
	SS2	3803265	3791275	3797270	97,61	1073,70			
	SS3	4495055	4922487	4708771	121,49	1336,39			
4 h	SS4	4070103	4061669	4065886	104,65	1151,12	1238,08	113,30	9,15
	SS5	4214360	4235291	4224826	108,81	1196,92			
	SS6	4806253	4818209	4812231	124,20	1366,21			
8 h	SS7	4471578	4901315	4686447	120,91	1329,96	1279,42	44,59	3,48
	SS8	4394578	4393264	4393921	113,24	1245,66			
	SS9	4451298	4454472	4452885	114,79	1262,65			
12 h	SS10	4245077	4238776	4241927	109,26	1201,85	1260,49	85,07	6,75
	SS11	4282899	4337781	4310340	111,05	1221,57			
	SS12	4756659	4811269	4783964	123,46	1358,07			
24 h	SS13	4439873	4444794	4442334	114,51	1259,61	1248,26	17,94	1,44
	SS14	4436449	4434311	4435380	114,33	1257,60			
	SS15	4323200	4339184	4331192	111,60	1227,58			
48 h	SS16	4412594	4401510	4407052	113,59	1249,44	1265,47	14,25	1,13
	SS17	4475427	4483237	4479332	115,48	1270,27			
	SS18	4503611	4499757	4501684	116,06	1276,71			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **Fin. Conc.**: Final Concentration, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering**: **S**: Sulfadoxine, **S**: SBS buffer solution, **1 – 18**: Sample number.

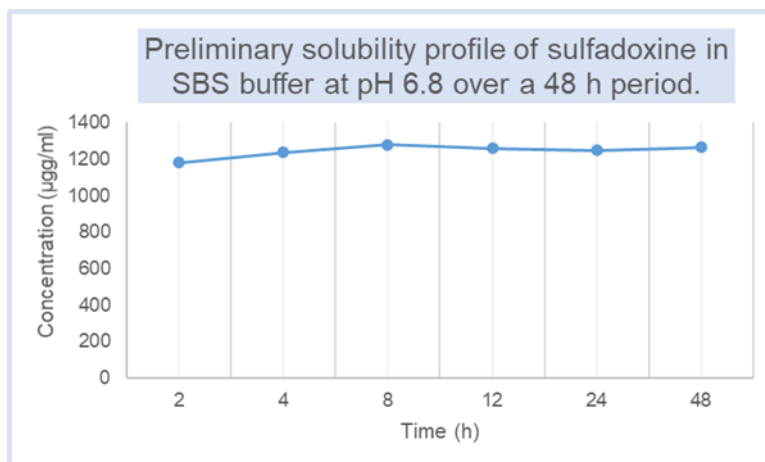


Figure 4.45: Preliminary solubility profile of sulfadoxine (single component) in SBS buffer solution.

It is evident from the data obtained that sulfadoxine entered into solution rapidly and remained stable in solution during 48 h. It had a maximum solubility of approximately 1279 µg/ml at 8 h. For each time interval, the average concentration was in close range to one another, therefore equilibrium was attained at 2 h and maintained for the 48 h period.

Preliminary solubility results obtained for sulfadoxine (S + P combination) in SBS buffer solution are presented in Table 4.43 and Figure 4.46.

Table 4.43: Preliminary solubility data of sulfadoxine in the S + P combination in SBS buffer solution at pH 6.8

Preliminary - Sulfadoxine (S+P combination) in SBS									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPS1	4632925	4286835	4459880	114,97	1149,70	1170,44	29,76	2,54
	SPS2	4485024	4853390	4669207	120,45	1204,54			
	SPS3	4677208	4299019	4488114	115,71	1157,09			
4 h	SPS4	4254384	4268138	4261261	109,77	1097,66	1118,06	20,12	1,80
	SPS5	4344064	4338584	4341324	111,86	1118,63			
	SPS6	4450195	4379352	4414774	113,79	1137,88			
8 h	SPS7	4952058	4928738	4940398	127,56	1275,59	1282,2	37,28	2,91
	SPS8	4833513	4841809	4837661	124,87	1248,67			
	SPS9	4881210	5356473	5118842	132,23	1322,34			
12 h	SPS10	4765015	5010036	4887526	126,17	1261,74	1270,15	19,22	1,51
	SPS11	5182177	4824983	5003580	129,21	1292,14			
	SPS12	4885812	4849821	4867817	125,66	1256,57			
24 h	SPS13	5097939	4734108	4916024	126,92	1269,20	1257,03	20,01	1,59
	SPS14	4929627	4892801	4911214	126,79	1267,94			
	SPS15	4796909	4765916	4781413	123,39	1233,94			
48 h	SPS16	5175152	5268737	5221945	134,94	1349,36	1282,97	66,34	5,17
	SPS17	5016496	4919892	4968194	128,29	1282,87			
	SPS18	4715472	4715638	4715555	121,67	1216,68			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering: S:** Sulfadoxine, **P:** Pyrimethamine, **S:** SBS buffer solution, **1 – 18:** Sample number.

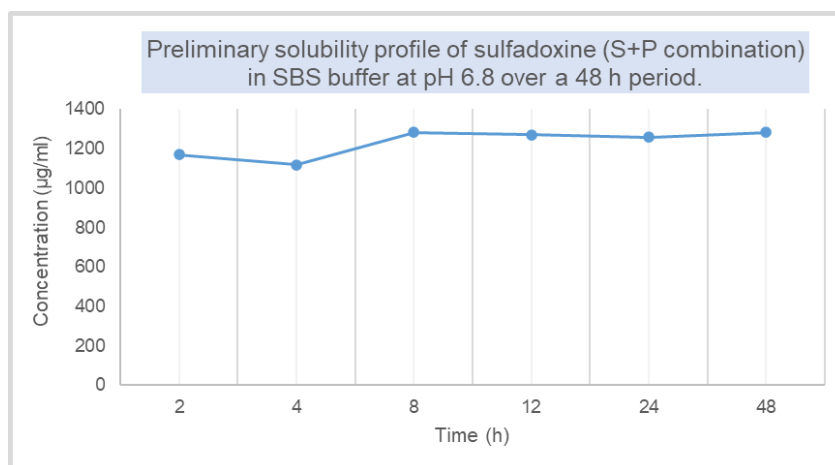


Figure 4.46: Preliminary solubility profile of artesunate (in A + P combination) in SBS buffer solution.

The data presented in Figure 4.46 indicated a similar solubility profile of sulfadoxine when combined with pyrimethamine than compared to the single component. Sulfadoxine demonstrated a rapid solubility and maintained stability throughout the 48 h study period. Specifically, in the S + P combination, the maximum solubility of sulfadoxine was approximately 1282 µg/ml at 8 h, where after equilibrium was also reached.

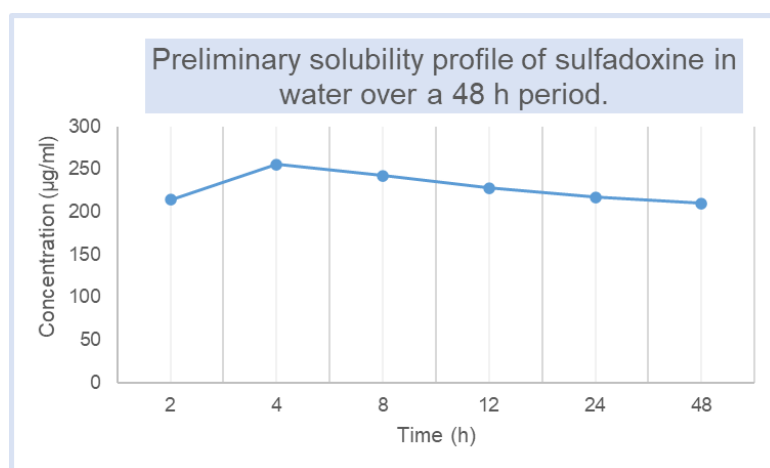
4.3.3.6 Preliminary solubility in water

Preliminary solubility results obtained for sulfadoxine (single component) in ultra-purified water are presented in Table 4.44 and Figure 4.47.

Table 4.44: Preliminary solubility data of sulfadoxine in water

Preliminary - Sulfadoxine (Single) in water								
Time	Sample	AUC 1	AUC 2	AVG.	Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SW1	8882376	8863563	8872970	231,48	214,29	15,64	7,30
	SW2	8061461	8075256	8068359	210,49			
	SW3	7690864	7710664	7700764	200,90			
4 h	SW4	9641376	9637815	9639596	251,48	255,80	12,77	4,99
	SW5	9422913	9416858	9419886	245,75			
	SW6	10353169	10359347	10356258	270,17			
8 h	SW7	8246583	9089628	8668106	226,13	242,18	18,91	7,81
	SW8	9099858	9097912	9098885	237,37			
	SW9	10090302	10074036	10082169	263,02			
12 h	SW10	7936078	7876872	7906475	206,27	228,33	30,57	13,39
	SW11	8258481	8262621	8260551	215,50			
	SW12	10109724	10070479	10090102	263,23			
24 h	SW13	8455143	8474161	8464652	220,83	217,00	3,38	1,56
	SW14	8243899	8298581	8271240	215,78			
	SW15	8244980	8191224	8218102	214,40			
48 h	SW16	7867842	7845859	7856851	204,97	210,04	7,48	3,56
	SW17	7914810	7917512	7916161	206,52			
	SW18	8377278	8383132	8380205	218,62			

***AUC**: Area under the curve, **AVG**: Average of the area under the curve, **Conc**: Concentration in µg/ml, **AVG. Conc**: Average concentration in µg/ml, **SD**: Standard deviation of the concentration per time interval, **%RSD**: Relative standard deviation (%) **Sample numbering: S**: Sulfadoxine, **W**: water, **1 – 18**: Sample number.

**Figure 4.47:** Preliminary solubility profile of sulfadoxine (single component) in water.

It is clear from the results above that sulfadoxine dissolved quickly and attained a maximum solubility of $\pm 255 \mu\text{g/ml}$ within 4 h after which a slight decrease was observed in the concentration throughout the 48 h period.

Preliminary solubility results obtained for sulfadoxine (S + P combination) in ultra-purified water are presented in Table 4.45 and Figure 4.48.

Table 4.45: Preliminary solubility data of sulfadoxine in the S + P combination in water

Preliminary - Sulfadoxine (S+P Combination) in water									
Time	Sample	AUC 1	AUC 2	AVG.	Conc.	Fin. Conc. (µg/ml)	AVG. Conc. (µg/ml)	SD	%RSD
2 h	SPW1	1630696	1631561	1631129	42,57	425,68	417,81	14,66	3,51
	SPW2	1535796	1536422	1536109	40,09	400,89			
	SPW3	1635768	1635566	1635667	42,69	426,86			
4 h	SPW4	1593077	1597375	1595226	41,63	416,31	412,85	4,35	1,05
	SPW5	1589385	1585349	1587367	41,43	414,26			
	SPW6	1561518	1564945	1563232	40,80	407,97			
8 h	SPW7	1745646	1761036	1753341	45,76	457,56	464,08	15,80	3,40
	SPW8	1767373	1927453	1847413	48,21	482,10			
	SPW9	1729481	1739116	1734299	45,26	452,59			
12 h	SPW10	1669321	1657935	1663628	43,42	434,15	443,31	24,10	5,44
	SPW11	1625234	1632779	1629007	42,51	425,12			
	SPW12	1725520	1881545	1803533	47,07	470,65			
24 h	SPW13	1710001	1706383	1708192	44,58	445,78	451,13	5,61	1,24
	SPW14	1673909	1828266	1751088	45,70	456,97			
	SPW15	1804113	1649431	1726772	45,06	450,63			
48 h	SPW16	1973289	1784276	1878783	49,03	490,28	470,95	18,01	3,82
	SPW17	1754208	1730173	1742191	45,46	454,65			
	SPW18	1710282	1875903	1793093	46,79	467,93			

***AUC:** Area under the curve, **AVG:** Average of the area under the curve, **Conc:** Concentration in µg/ml, **AVG. Conc:** Average concentration in µg/ml, **SD:** Standard deviation of the concentration per time interval, **%RSD:** Relative standard deviation (%) **Sample numbering:** **S:** Sulfadoxine, **P:** Pyrimethamine, **W:** water, **1 – 18:** Sample number.

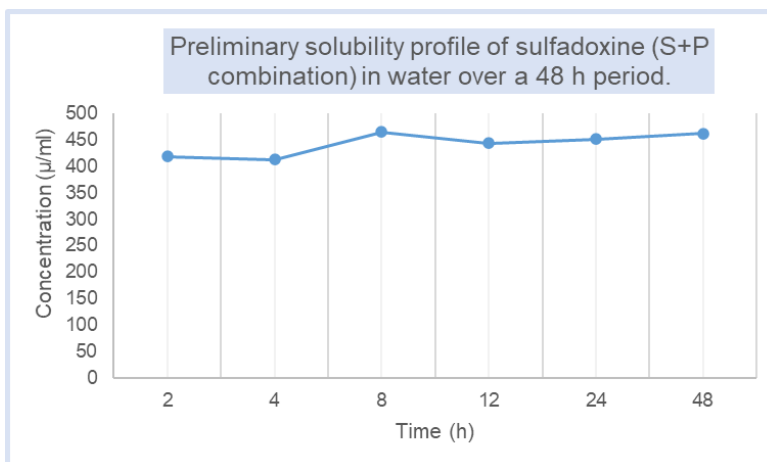


Figure 4.48: Preliminary solubility profile of sulfadoxine (in S + P combination) in water.

It is clear from Figure 4.48 that the solubility concentration of sulfadoxine in the presence of pyrimethamine almost doubled. Sulfadoxine rapidly solubilised and maintained stability during the 48 h study. The maximum solubility of sulfadoxine in S + P combination was $\pm 470 \mu\text{g/ml}$ at 48 h.

4.3.3.7 Pivot solubility of sulfadoxine as single component and sulfadoxine in S + P combination

In Table 4.46 and Figure 4.49 below, is a compilation of the pivot solubility studies performed for sulfadoxine single component and in combination with pyrimethamine in the various solubility media ranging from a pH 1.2 – 6.8. Pivot solubility studies for sulfadoxine was performed at 2 h due to rapid achievement of equilibrium proved in the preliminary studies.

Table 4.46: Pivot solubility of sulfadoxine in single component and S + P combination in various media.

Solvent	Average Concentration ($\mu\text{g/ml}$)					
	Sulfa (single)	SD	%RSD	Sulfa (S+P Combo)	SD	%RSD
0.1 N HCl	995,90	1,81	0,18	666,20	0,35	0,05
NaCl	931,10	0,79	0,09	719,68	0,32	0,04
ACE	234,00	1,59	0,68	278,21	9,71	3,49
PBS	1595,41	1,92	0,12	1321,49	4,19	0,32
SBS	1365,86	0,84	0,06	1208,84	3,36	0,28
Water	246,69	20,43	8,28	463,96	1,39	0,30

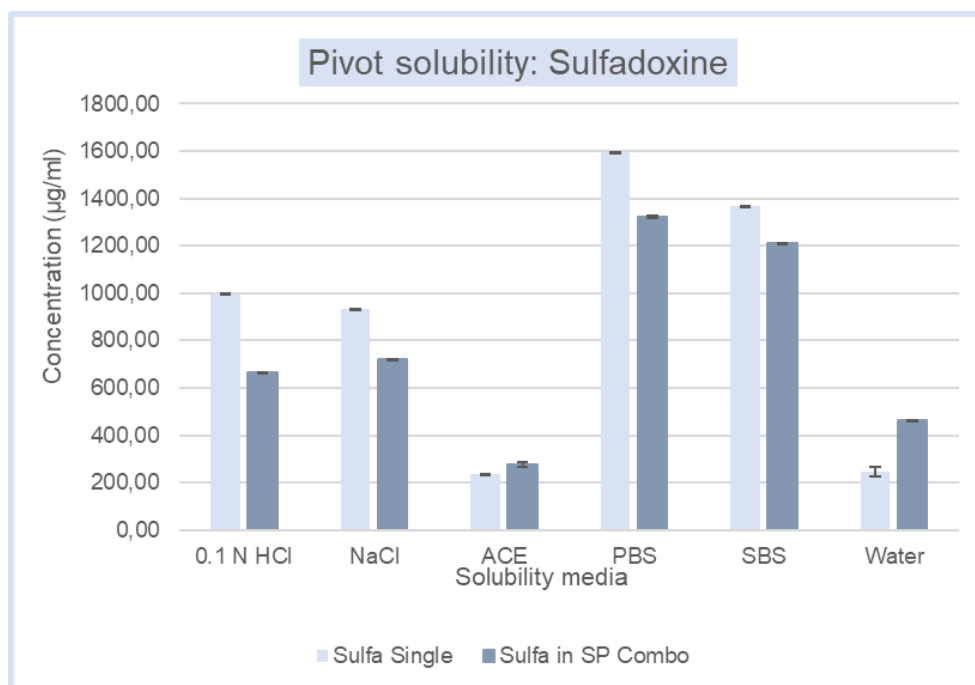


Figure 4.49: Pivot solubility of sulfadoxine in single component and S + P combination in various media.

The data obtained, as seen in Figure 4.46 and Table 4.49, indicate only an enhancement in the solubility of sulfadoxine when combined with pyrimethamine in the ACE buffer and in water. Sulfadoxine in the single component mainly showed a higher solubility concentration, thus it can be inferred that sulfadoxine was not greatly affected by the presence of pyrimethamine. Remarkably, the solubility for sulfadoxine in combination with pyrimethamine in water doubled. This correlates with the study performed by [Badenhorst \(2017\)](#).

4.4 Statistical analysis

4.4.1 ANCOVA analysis of preliminary studies

An analysis of covariance (ANCOVA) was used to determine the difference between the two solubility profiles of each single component and its relevant combination on a statistically significant level. For this study, the assumption of parallel regression lines was foregone, and the following model (Eq. 1) was selected:

$$y_{ij} = \alpha_i + \beta_i x_{ij} + \epsilon_{ij}; j = 1, \dots, n_i; i = 1, 2; \quad \text{Eq. 1}$$

where ϵ_{ij} are independent $n(0; \sigma^2)$ variates.

In this model, we estimated (α_1, β_1) and (α_2, β_2) independently (Eq.'s 2 - 3), but σ^2 jointly as Eq. 4:

$$\hat{\beta}_i = \frac{\sum_j y_{ij}(x_{ij} - \bar{x}_i)}{\sum_j (x_{ij} - \bar{x}_i)^2} \quad \text{Eq. 2}$$

$$\hat{\alpha}_i = \bar{y}_i - \hat{\beta}_i \bar{x}_i \quad \text{Eq. 3}$$

$$\hat{\sigma}^2 = \frac{\sum_i \sum_j (y_{ij} - \hat{\alpha}_i - \hat{\beta}_i x_{ij})^2}{(n_1 + n_2 - 4)} \quad \text{Eq. 4}$$

The responses at time zero and at time n_i , were tested pairwise for the solubility data to see if they were equal. Therefore, the hypothesis tested for the solubility data was $H_0: \alpha_1 + \beta_1 x = \alpha_2 + \beta_2 x$, compared to the test statistic:

$$f_x = (\hat{\alpha}_1 + \hat{\beta}_1 x - \hat{\alpha}_2 - \hat{\beta}_2 x)^2 / \hat{\sigma}^2 \left[\frac{1}{n_1} + \frac{(x - \bar{x}_1)^2}{\sum (x_{1j} - \bar{x}_1)^2} + \frac{1}{n_2} + \frac{(x - \bar{x}_2)^2}{\sum (x_{2j} - \bar{x}_2)^2} \right] \quad \text{Eq. 5}$$

with $F_{\alpha; 1; n_1 + n_2 - 4}$. Testing at $\alpha = 0.05$, any values of $f_x > F_{crit}$ will therefore be indicative of a statistically significant difference.

Artesunate: Table 4.47 presents the ANCOVA test statistics for artesunate and the A + P combination. The F_{crit} value at the 5.0% level of significance is 5.3177.

Table 4.47: ANCOVA statistics of artesunate and A + P combination

	ART HCl	AP HCl
ART HCl		36,02
AP HCl	36,02	

	ART NaCl	AP NaCl
ART NaCl		7,5367
AP NaCl	7,5367	

	ART ACE	AP ACE
ART ACE		46,0127
AP ACE	46,0127	
	ART PBS	AP PBS
ART PBS		62,4003
AP PBS	62,4003	
	ART SBS	AP SBS
ART SBS		68,6412
AP SBS	68,6412	
	ART H₂O	AP H₂O
ART H₂O		205,3536
AP H₂O	205,3536	

***ART:** Artesunate, **AP:** Artesunate-Pyrimethamine, **HCl:** HCl solution, **NaCl:** Sodium chloride buffer, **ACE:** Acetate buffer, **PBS:** potassium phosphate buffer, **SBS:** Sodium phosphate buffer, **H₂O:** Water

From the data seen in Table 4.47 above, it can be concluded that there was a significant difference in the solubility profiles between artesunate and the A + P combination, since all the values were above the F_{crit} value (5.3177).

Pyrimethamine: In Table 4.48 is the ANCOVA statistics of pyrimethamine and the A + P combination. The F_{crit} value at the 5.0% level of significance is 5.3177.

Table 4.48: ANCOVA statistics of pyrimethamine and A + P combination

	PYRI HCl	PA HCl
PYRI HCl		12,913
PA HCl	12,913	

	PYRI NaCl	PA NaCl
PYRI NaCl		16,5753
PA NaCl	16,5753	

	PYRI ACE	PA ACE
PYRI ACE		29,9019
PA ACE	29,9019	
	PYRI PBS	PA PBS
PYRI PBS		30,7032
PA PBS	30,7032	
	PYRI SBS	PA SBS
PYRI SBS		14,9115
PA SBS	14,9115	
	PYRI H₂O	PA H₂O
PYRI H₂O		223,883
PA H₂O	223,883	

***PYRI:** Pyrimethamine, **PA:** Pyrimethamine-artesunate, **HCl:** HCl solution, **NaCl:** Sodium chloride buffer, **ACE:** Acetate buffer, **PBS:** potassium phosphate buffer, **SBS:** Sodium phosphate buffer, **H₂O:** Water

From Table 4.48 above, it is clear that there was a significant difference in the solubility profiles between pyrimethamine and the A + P combination, since all the values for all the solubility media are above the F_{crit} value (5.3177).

Pyrimethamine: The ANCOVA statistics of pyrimethamine and the S + P combination are presented in Table 4.49. The F_{crit} value at the 5.0% level of significance is 5.3177.

Table 4.49: ANCOVA statistics of pyrimethamine and S + P combination

	PYRI HCl	PS HCl
PYRI HCl		11,0481
PS HCl	11,0481	

	PYRI NaCl	PS NaCl
PYRI NaCl		5,0936
PS NaCl	5,0936	

	PYRI ACE	PS ACE
PYRI ACE		41,1484
PS ACE	41,1484	

	PYRI PBS	PS PBS
PYRI PBS		88,3516
PS PBS	88,3516	

	PYRI SBS	PS SBS
PYRI SBS		125,7565
PS SBS	125,7565	

	PYRI H2O	PS H2O
PYRI H2O		694,176
PS H2O	694,176	

***PYRI:** Pyrimethamine, **PS:** Pyrimethamine-sulfadoxine, **HCl:** HCl solution, **NaCl:** Sodium chloride buffer, **ACE:** Acetate buffer, **PBS:** potassium phosphate buffer, **SBS:** Sodium phosphate buffer, **H2O:** Water

It is clear that there was a significant difference in the solubility profiles between pyrimethamine and the S + P combination, since all the values are above the F_{crit} value (5.3177), except the solubility profiles of pyrimethamine and the S + P combination in the NaCl buffer solution.

Sulfadoxine: In Table 4.50 are the statistics regarding sulfadoxine and the S + P combination. The F_{crit} value at the 5.0% level of significance is 5.3177.

Table 4.50: ANCOVA statistics of sulfadoxine and S + P combination

	SULFA HCl	SP HCl
SULFA HCl		70,7077
SP HCl	70,7077	

	SULFA NaCl	SP NaCl
SULFA NaCl		10,7048
SP NaCl	10,7048	

	SULFA ACE	SP ACE
SULFA ACE		117,6196
SP ACE	117,6196	

	SULFA PBS	SP PBS
SULFA PBS		1,6648
SP PBS	1,6648	

	SULFA SBS	SP SBS
SULFA SBS		8,7113
SP SBS	8,7113	

	SULFA H ₂ O	SP H ₂ O
SULFA H ₂ O		193,738
SP H ₂ O	193,7376	

***SULFA:** Sulfadoxine, **SP:** Sulfadoxine-pyrimethamine, **HCl:** HCl solution, **NaCl:** Sodium chloride buffer, **ACE:** Acetate buffer, **PBS:** potassium phosphate buffer, **SBS:** Sodium phosphate buffer, **H₂O:** Water

It can be concluded that there was a significant difference in the solubility profile between sulfadoxine and the S + P combination since all the values are above the F_{crit} value (5.3177) for the 0.1 N HCl solution, the NaCl-, ACE-, and SBS buffer solution, and for water. However, the difference between the solubility profiles of sulfadoxine and the S + P combination in the PBS buffer solution was not significant since the F_{crit} value was 1.6648.

4.4.2 ANOVA analysis for pivot studies

Boxplots of the pivot solubility data obtained from this study are presented in Figure 4.50. Visual inspection suggests that the highest aqueous solubility values were observed for artesunate and the artesunate-pyrimethamine combination in both PBS and SBS. The solubility values of pyrimethamine and the pyrimethamine-sulfadoxine combination in acetate buffer were also higher than those of the other groups. However, it is important that the resultant concentration of the combination was found to be lower than that of the single component.

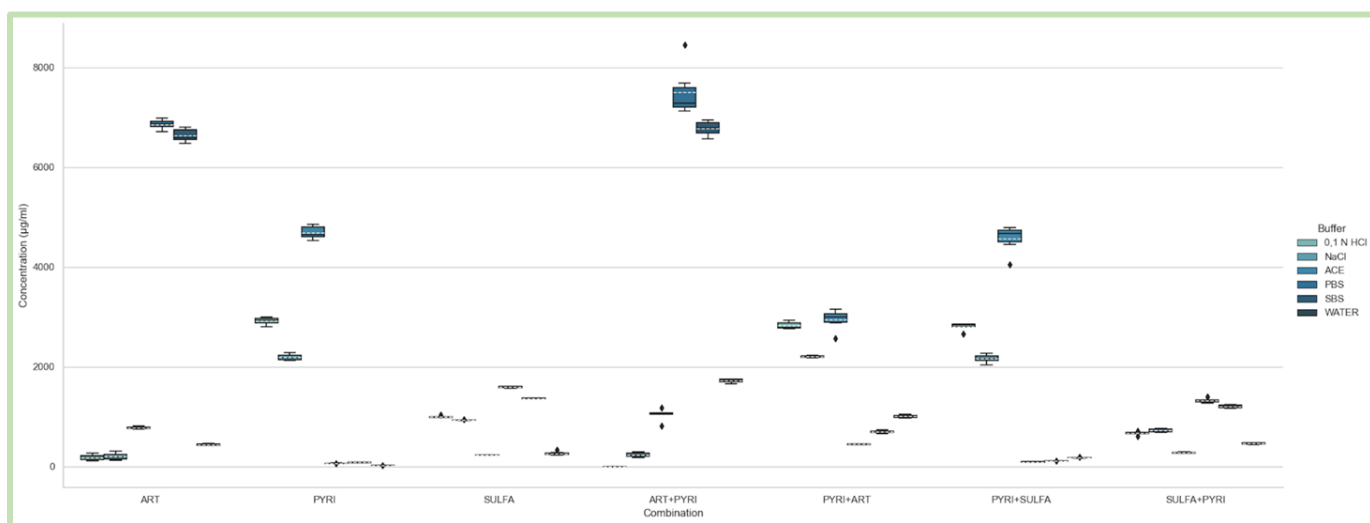


Figure 4.50: Boxplots of the pivot solubility study results obtained from the drug combinations and different buffer solutions tested in this study.

The ANOVA table of the results obtained from the pivot solubility studies is presented in Table 4.51.

Table 4.51: ARTANOVA table of the results obtained from the pivot solubility studies

Groups	Df	Df res	Sum of Squares	Sum of Squares res	F value	Pr(>F)	Partial η^2
Combination	6	210	1220715,11	101543,17	420,76	4,42e ⁻¹¹⁴	0,923
Buffer	5	210	1273509,57	53414,17	1001,37	2,48e ⁻¹⁴⁴	0,960
Combination: Buffer	30	210	1292122,94	5042,17	1793,84	4,56e ⁻²³⁶	0,996

The ANOVA results suggest that at least one treatment in each group differed from the rest at a statistically significant level. The largest effect size was observed for the interaction between the drug combination and buffer solution. Concretely, 99.6% of the variance in the ARTANOVA model could be explained by the interaction between the API combination and the buffer solution.

Results from the *post hoc* tests in Table 4.51 on the API combination group showed that the only treatments that did not differ from each other, at a statistically significant level ($p < 0.05$), was the pyrimethamine – pyrimethamine-sulfadoxine pair. Within the buffer group, all the treatments differed from each other at a statistically significant degree ($p < 0.05$). For the interaction between the drug combination and buffer solution group, six treatments differed significantly ($p < 0.05$) from all the other treatments but not from each other. These six treatments were artesunate in PBS, artesunate in SBS, artesunate in water, pyrimethamine in acetate buffer, pyrimethamine in water, artesunate-pyrimethamine in PBS, artesunate-pyrimethamine in SBS, artesunate-pyrimethamine in water, pyrimethamine-sulfadoxine in acetate buffer, and pyrimethamine-sulfadoxine in water.

4.5 Physico-chemical studies

4.5.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) involves the process where heat is released and then quantified. It can either be during a reaction or conformational alterations. When a change in the enthalpy ($\Delta T H$) is observed, it can be due to a chemical reaction occurring. When $\Delta T H$ is negative in value, the reaction is exothermic and releases heat; and when $\Delta T H$ is positive in value, the reaction is endothermic and requires additional heat for the process (Gill *et al.*, 2010; Mettler Toledo, 2000).

4.5.1.1 Artesunate-pyrimethamine combination

In Figure 4.51 shown below, the differential thermogram of artesunate (blue), pyrimethamine (red), and artesunate-pyrimethamine (AP) combination (black) is presented by the heat flow of the APIs as a function of temperature in °C. The combination experiment was performed in a 1:1 ratio.

Pyrimethamine (red): It is clear from this figure that pyrimethamine exhibits an endothermic reaction, and peaks at 235.98 °C. This correlates to the melting point reported by the International Pharmacopoeia, as stated in Chapter 2 Subsection 2.2.2 ([Ph. Int, 2022a](#)).

Artesunate (blue): Artesunate presents with an initial endothermic reaction, with a peak melting point at 146.65 °C. This correlates with the melting point reported by the American Chemical Society ([ACS, 2023](#)) as stated in Chapter 2 Subsection 2.2.1. However, artesunate is followed by another greater peak that is exothermic of nature, with an onset temperature of 158.90°C and peak temperature of 172.09°C. This can be degradation products or decomposition, notifiable, it is highly correlating to the DSC thermogram of dihydroartemisinin in a study done by Ansari *et al.*, (2011). In this study, dihydroartemisinin exhibited an exothermic reaction with an onset temperature of 159.20°C, and a peak temperature at 170.0°C ([Ansari *et al.*, 2011](#)). Artesunate is a pro-drug for the active metabolite “dihydroartemisinin” through hydrolyses in the liver ([Benakis *et al.*, 1997](#)).

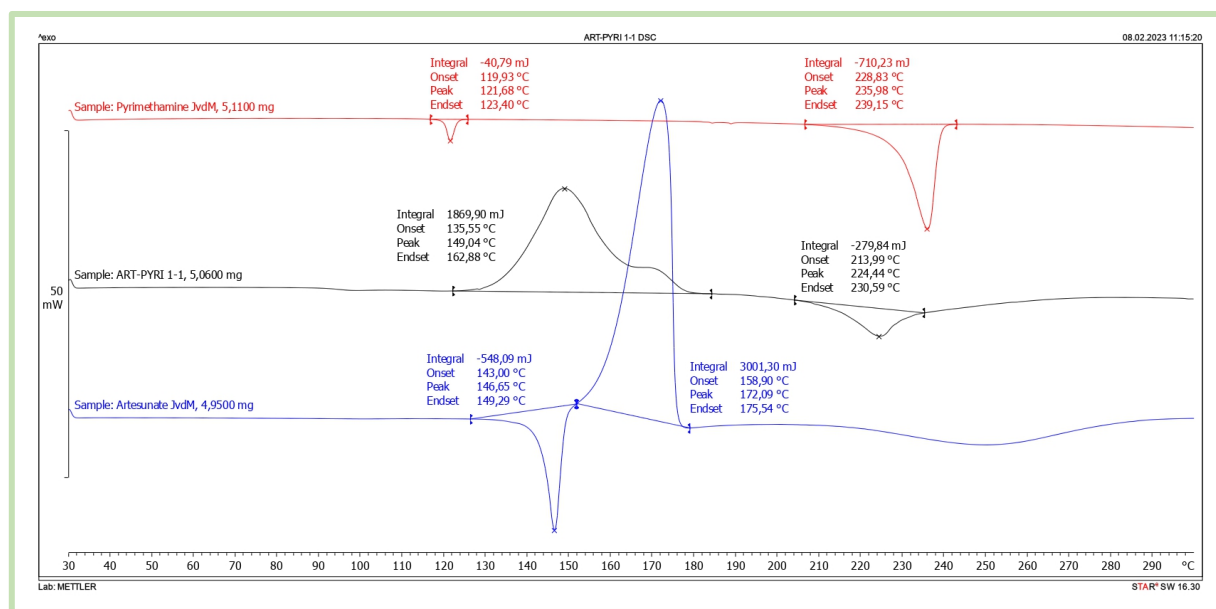


Figure 4.51: DSC thermogram of pyrimethamine (red), the 1:1 weight ratio of A + P combination (black) and artesunate (blue).

Artesunate-pyrimethamine combination (black): In the 1:1 combination there are two peaks observed. The first peak is an exothermic peak with a melting point of $\pm 149.04^{\circ}\text{C}$. This exothermic peak is the decomposition of artesunate that overlap the endothermic artesunate peak, which can be an incompatibility. The second peak is endothermic, which can be identified as pyrimethamine. However, the melting point is a little bit lower (224.44°C). Further investigation is needed to confirm these conclusions.

4.5.1.2 Pyrimethamine-sulfadoxine combination

In Figure 4.52 shown below, the differential thermogram of pyrimethamine (blue), sulfadoxine (red), and sulfadoxine-pyrimethamine (SP) combination (black) is presented by the heat flow of the APIs as a function of temperature in $^{\circ}\text{C}$. The combination experiment was performed in a 1:1 ratio.

Sulfadoxine (Red): It is clear from this figure that sulfadoxine exhibits a sharp melting endothermic peak at 197.13°C , after which a secondary exothermic peak is observed. The secondary peak at 260.03°C , is decomposition of sulfadoxine, which correlates to the data reported by the International Pharmacopoeia, as stated in Chapter 2 Subsection 2.2.3 ([Ph. Int. 2022b](#)).

Pyrimethamine (blue): Refer to subsection 4.4.1.1, where a complete description for pyrimethamine is given.

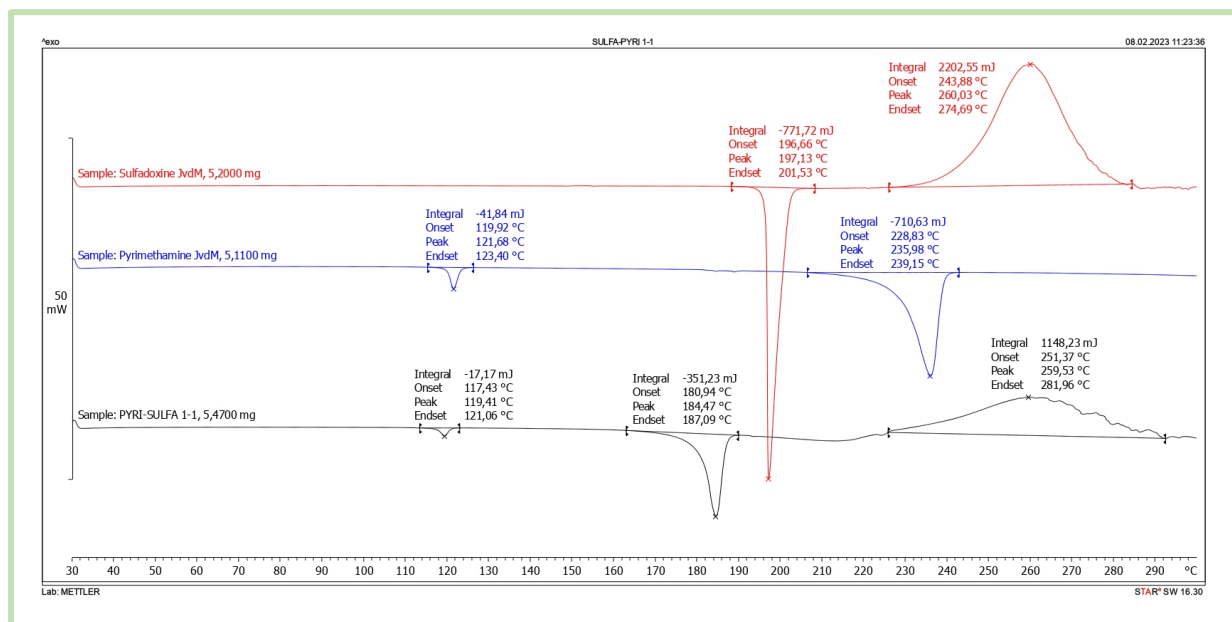


Figure 4.52: DSC thermogram of sulfadoxine (red), pyrimethamine (blue), and the 1:1 weight ratio of S + P combination (black).

Pyrimethamine-sulfadoxine combination: From this Figure 4.52, it can be seen that sulfadoxine and pyrimethamine melts within close range to one another. When combined, there is an endothermic peak with a melting point of 184.47°C, that is slightly lower than both sulfadoxine and pyrimethamine. This can possibly be a eutectic mixture forming ([Habib et al., 2001](#)). The decomposition of sulfadoxine (259.53°C) overshadow the pyrimethamine peak.

4.5.2 Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR), is when IR radiation pass through a sample, where radiation may either get absorbed by the sample or pass through. The extent of absorption depends on the functional groups (what the sample consist of) and frequencies of the vibrations between the different bonds and their respective angles. This presents then as a spectrum of absorption and transmission, and leaves a “fingerprint” identifiable by the specific sample ([Khan et al., 2018](#)).

4.5.2.1 Artesunate-pyrimethamine combination

In Figure 4.53 shown below, the FT-IR spectra of pyrimethamine (red), artesunate (black), and artesunate – pyrimethamine (A + P) combination (green) are presented. The combination experiment was performed in a 1:1 ratio.

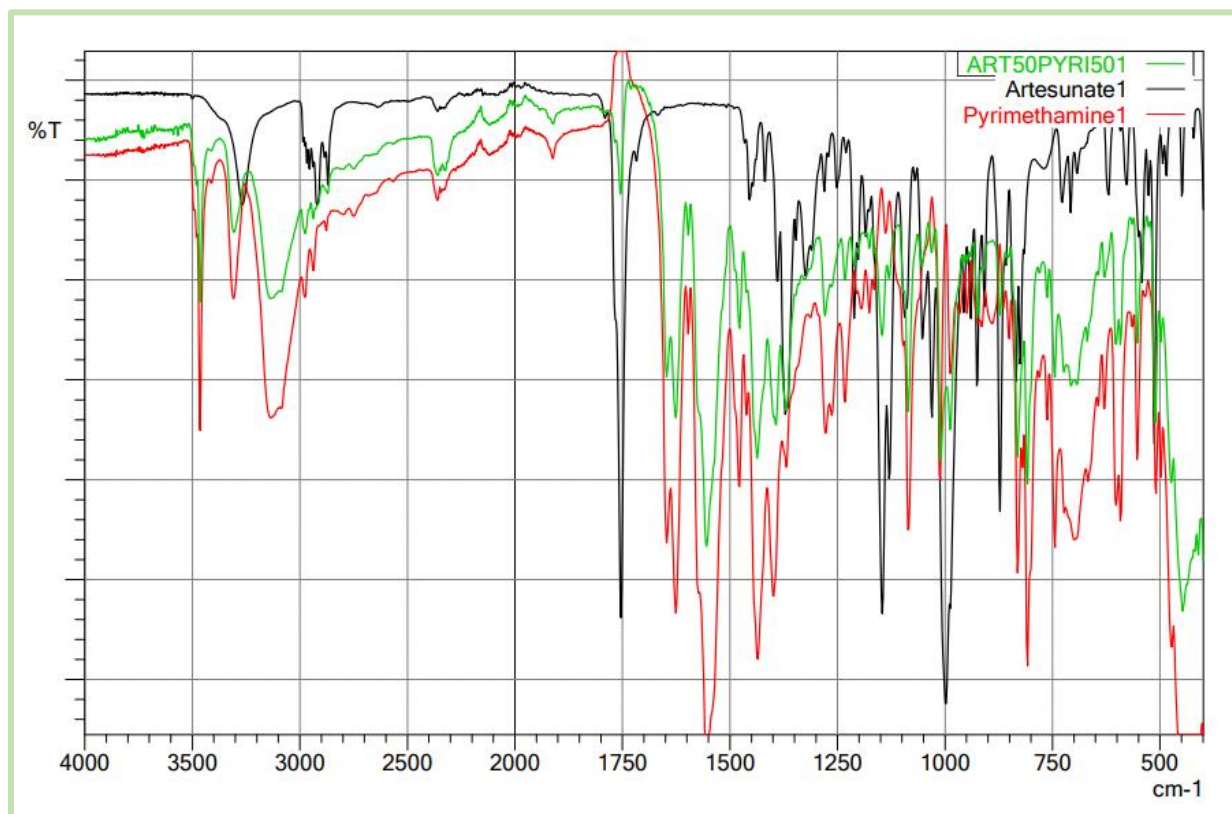


Figure 4.53: FT-IR spectra of artesunate, pyrimethamine, and artesunate-pyrimethamine (1:1 weight) combination.

Artesunate – pyrimethamine (green): In this experimental procedure, the same amount of both powders was combined and stirred before being applied to the FT-IR for analysis. The resulting spectrum for the A + P combination, distinctly reveals the functional groups associated with pyrimethamine API. In contrast, the analysis of artesunate indicates the complete disappearance of the hydroxyl group ($\pm 3300\text{ cm}^{-1}$), alkyl group ($\pm 2700 - 3000\text{ cm}^{-1}$), and carbonyl group ($\pm 1750\text{ cm}^{-1}$). This suggests a complete dispersion of artesunate within the pyrimethamine. Notably, this dispersion resulted in an insufficient presence of artesunate crystals to induce melting, as evidenced by the absence of characteristic features in the DSC thermogram, as discussed in subsection 4.5.1.1.

4.5.2.2 Pyrimethamine-sulfadoxine combination

In Figure 4.54 shown below, the FT-IR spectra of pyrimethamine (red), sulfadoxine (black), and sulfadoxine – pyrimethamine (S + P) combination (green) are presented. The combination experiment was performed in a 1:1 ratio.

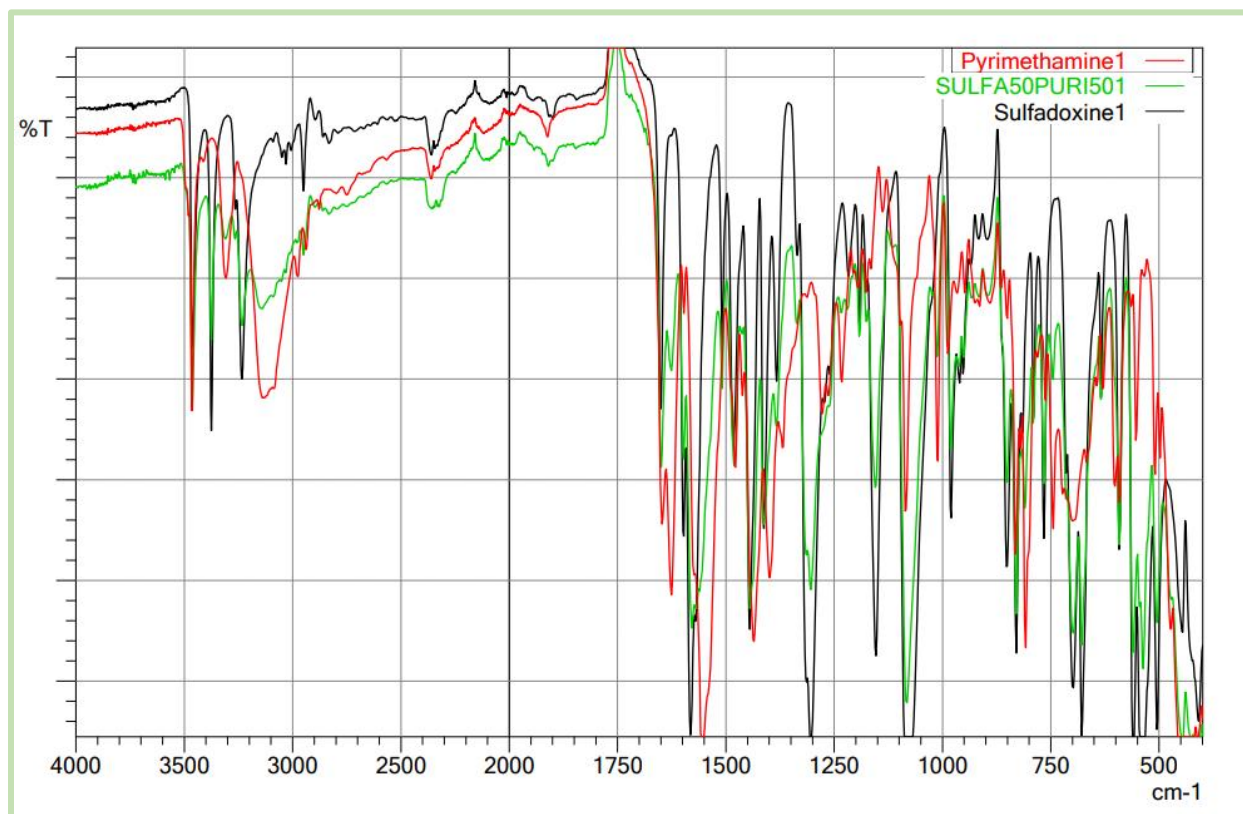


Figure 4.54: FT-IR spectra of pyrimethamine, sulfadoxine, and 1:1 weight ratio of S + P combination.

Pyrimethamine – sulfadoxine (green): In this experimental process, equal amounts the powders were combined and stirred before being applied to the FT-IR for analysis. The resulting spectrum for the S + P combination, distinctly elucidates the functional groups associated with each respective API. This observation implies that there is no noticeable interaction between the two APIs.

4.5.3 Thermal Activity Microcalorimetry (TAM)

Microcalorimetry is used to study possible chemical interactions between APIs. Microcalorimetry is very sensitive when measuring the heat flow (evolved or absorbed) during a chemical process that gives a framework of the rate and extent to which reaction take place ([Koenigbauer, 1994](#)). For this study TAM was set at 40°C. The results are discussed below.

4.5.3.1 Artesunate – pyrimethamine combination

In the Figure 4.55 shown below, the TAM data of A + P combination are presented. The combination experiment was performed in a 1:1 ratio at 40°C. It can be inferred that the artesunate – pyrimethamine combination exhibits no noticeable interactions. The interaction integrals fall below the range of 2 – 3 J/g.

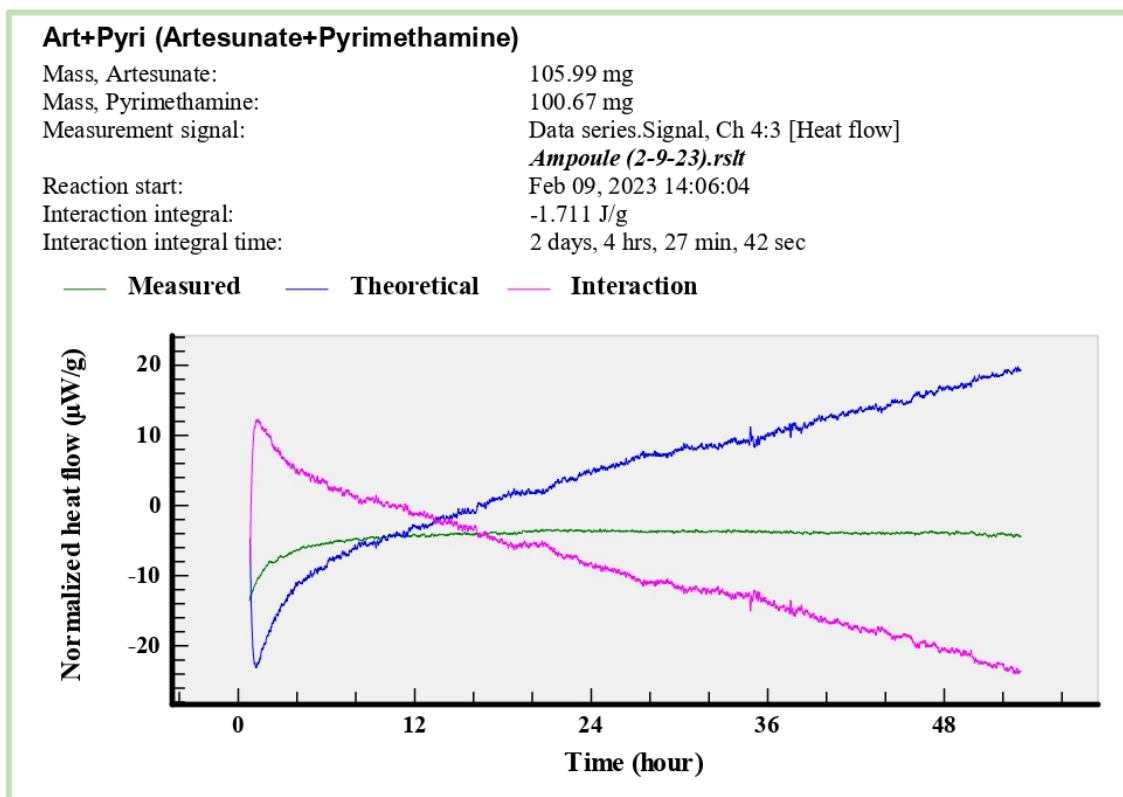


Figure 4.55: Thermal Activity Monitoring (TAM) of artesunate – pyrimethamine combination.

4.5.3.2 Pyrimethamine – sulfadoxine combination

In the Figure 4.56 shown below, the TAM data of S + P combination are presented. The combination experiment was performed in a 1:1 ratio. It can be inferred that the sulfadoxine – pyrimethamine combination exhibits no noticeable interactions. The interaction integrals fall below the range of 2 – 3 J/g.

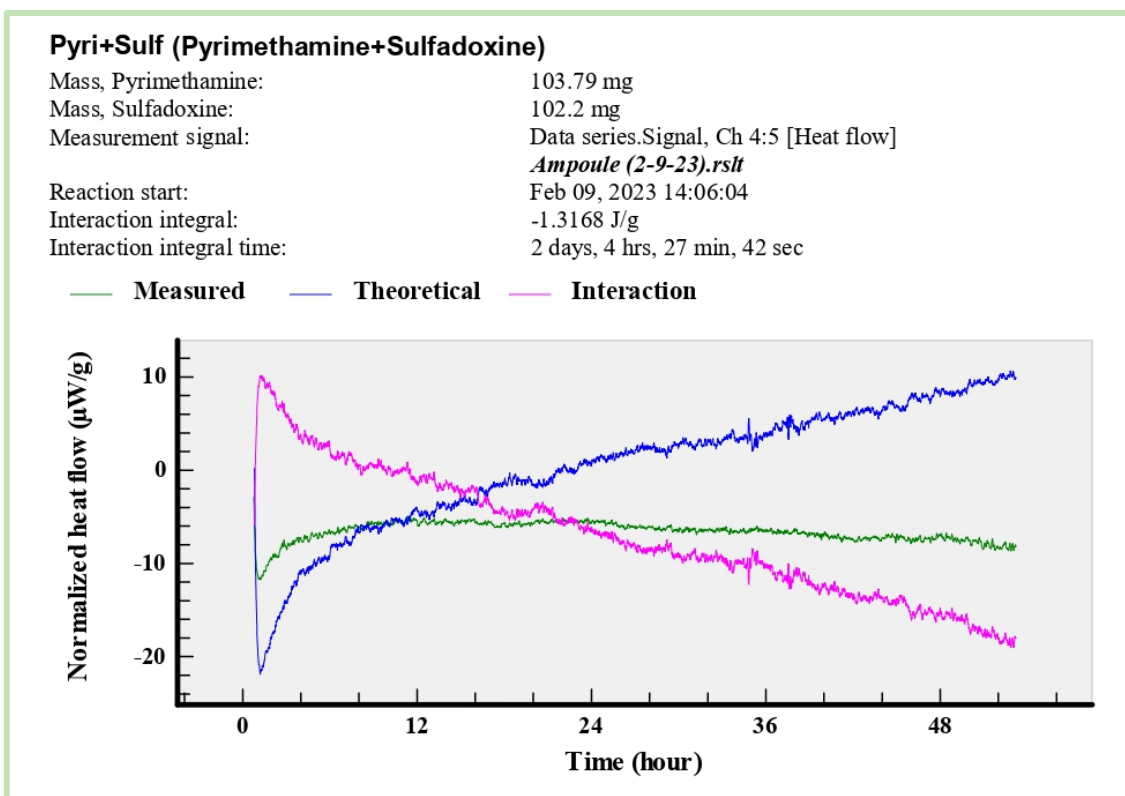


Figure 4.56: Thermal Activity Microcalorimetry (TAM) of pyrimethamine – sulfadoxine combination.

4.6 Conclusion

During validation, the analysis of each API was subjected to various parameters (linearity, specificity, precision, accuracy, LOD, and LOQ). Artesunate proved successful in all solutions/buffers except 0.1 N HCl solution, and NaCl buffer since rapid degradation at a low pH was apparent. For this reason, a single injection for each standard was used to obtain a calibration curve, from which the data analysis of solubility samples in these two media was performed. Pyrimethamine and sulfadoxine were analysed using the same method, and both proved successful regarding the parameters evaluated in the various buffers/solutions.

Pyrimethamine and sulfadoxine did, however, produce higher %RSD values for the lowest concentration standard, which can be attributed to the fact that this concentration was very close to the LOQ or LOD.

Preliminary. During the preliminary solubility studies of artesunate, it was found that the stability of the API depreciated as time passed, especially in the lower pH of 1.2. This was true for both single components and in combination with pyrimethamine, except for artesunate in combination with pyrimethamine in ACE buffer. Artesunate proved most stable in ACE buffer in combination with pyrimethamine. The difference in these solubility profiles was established with the ANCOVA statistical analysis, and the significance thereof was proven since all the F critical values were above 5.3177. This is confirmation that the presence of pyrimethamine improves the solubility of artesunate by increasing the surrounding pH due to its weakly basic nature, while simultaneously causing an increase in the degradation of artesunate as well.

For pyrimethamine (in combination with artesunate and the single component), the solubility profiles obtained proved stability in most of the solvents/buffers, with the exception of NaCl, where the API remained stable in combination for up to 24 hours after degradation was observed. Furthermore, pyrimethamine proved more stable in 0.1 N HCl compared to NaCl, despite the pH of both solvents being 1.2, which proves that there are solubility differences with regard to buffer composition. The same can be said for the solubility profiles of pyrimethamine (A + P combination) in PBS and SBS; equilibrium was achieved at 12 h and 24 h, respectively. Pyrimethamine in combination with sulfadoxine proved stable in all media tested and reached equilibrium early in the experiment, except for the combination in 0.1 N HCl, whereby the maximum solubility was only obtained at 12 hours. The significance of the differences between the solubility profiles was established with the ANCOVA statistical analysis since all the F critical values were above 5.3177.

Sulfadoxine proved stable in all media (both as a single component and in combination with pyrimethamine). For sulfadoxine in combination with pyrimethamine in SBS, equilibrium was only achieved at 8 hours. The ANCOVA analysis showed that the solubility profiles for the sulfadoxine and S + P combination in the PBS buffer were not significantly different, which was also seen throughout the 48 h of the preliminary study.

Pivot. Artesunate in combination with pyrimethamine increased as the pH increased, except for the 0.1 N HCl solution. The highest solubility was seen in the PBS buffer solution, compared

to all the other media, for the single component as well as in combination with pyrimethamine. It is important to note that the solubility of artesunate improved more than three-fold when in combination with pyrimethamine in water.

Pyrimethamine in combination with artesunate showed a substantial increase in solubilities with the higher pH levels of PBS (6-fold increase), SBS buffer solution (8-fold increase) at pH 6.8, and water (46-fold increase) in comparison to the single component. However, the highest solubility was seen in the ACE buffer solution for pyrimethamine (single) and the A+P combination, respectively, but when these two were compared to each other, pyrimethamine decreased when in combination with artesunate.

More or less the same trend as mentioned above was seen throughout the pH range of 1.2 – 6.8 when the pyrimethamine single component was compared to the S + P combination. Pyrimethamine in combination with sulfadoxine in water showed an 8-fold increase in solubility compared to the single component. In contrast, pyrimethamine in the S + P combination showed a decreased solubility in the ACE compared to the single component, even though both exhibited the highest solubilities, respectively, compared to the whole pH range.

Sulfadoxine in the presence of pyrimethamine was not greatly affected due to the single component exhibiting higher solubility profiles in 0.1 N HCl, NaCl, PBS, and SBS. Increased solubilities were only seen in water (doubled) and in the ACE buffer solution when sulfadoxine was combined with pyrimethamine.

Physico-chemical studies were additionally performed to give insight on possible interactions between the APIs. Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), and thermal activity microcalorimetry (TAM) tests were performed. TAM was done at a set temperature of 40°C. No interactions were observed in the results for both the A + P combination and the S + P combination.

During the FT-IR analysis of artesunate in combination with pyrimethamine, a complete disappearance of the hydroxyl group ($\pm 3300\text{ cm}^{-1}$), alkyl group ($\pm 2700 - 3000\text{ cm}^{-1}$), and carbonyl group ($\pm 1750\text{ cm}^{-1}$) was seen. This could be that artesunate is dispersed within the pyrimethamine. It is noteworthy that this dispersion resulted in an insufficient presence of artesunate crystals to induce melting, as verified by the absence of characteristic features in the DSC thermogram.

Furthermore, a possible eutectic mixture was presented during the DSC thermogram of the pyrimethamine-sulfadoxine combination. However, further studies need to be conducted to confirm these findings, whereas the FT-IR showed no possible interactions.

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CHAPTER 5: CONCLUSION

5.1 Conclusion

This study focused on addressing the rising global issue of malaria, specifically *P. falciparum* infections. A particular problem is the increasing resistance of the parasite against APIs in the African region. Recognising this, the World Health Organisation (WHO), advocates for the development and formulation of fixed dose combinations (FDCs) for malaria, with a specific emphasis on the combination artesunate, pyrimethamine, and sulfadoxine (WHO, 2021).

Prior to formulation, comprehensive research on the compatibility of different APIs and the interactions between them and their excipients is imperative. When different APIs are combined, factors such as stability, physico-chemical properties, solubility, and bioavailability must be thoroughly investigated. The main objective of this study was to concentrate on the solubility and physico-chemical characterisation of the three anti-malarial APIs, with particular focus on pyrimethamine and its compatibility with artesunate and sulfadoxine, since the information available is limited or, in certain instances, lacking. This necessitated an investigation in this regard.

High-performance liquid chromatography (HPLC) methods were validated and subsequently employed for the analysis of solubility studies. All three APIs were submitted to key parameters during validation, including precision, specificity, selectivity, linearity, and accuracy. All the APIs were successful in this regard, except for artesunate in pH 1.2 media (failing intermediate precision and repeatability) due to the rapid degradation in this particular medium. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the linearity data, further enhancing the analytical rigor of the study.

Solubility studies were conducted on the individual APIs and various combinations, encompassing a pH spectrum from 1.2 to 6.8. Pyrimethamine was the common focus for both combinations that were performed: artesunate with pyrimethamine and sulfadoxine with pyrimethamine. Preliminary studies explored the solubility profiles of the APIs and the combinations over 48 h, followed by pivot solubility studies withdrawn at 2 h.

Artesunate exhibited degradation throughout most of the media during the preliminary solubility studies, regardless of the single component or part of a combination. Notably, in the ACE buffer

at a pH of 4.5, artesunate remained relatively stable, particularly when in combination with pyrimethamine. Across the pH range of 1.2 to 6.8, the solubility of artesunate's (as a single component) showed the highest in PBS, followed by a decreasing order: PBS >> SBS >> ACE >> water >> NaCl >> 0.1 N HCl. This trend mostly held true during the combination with pyrimethamine, except for water showing higher solubility than ACE in the presence of pyrimethamine. Additionally, artesunate exhibited improved water solubility (three-fold) when in combination with pyrimethamine than as the single component.

During the solubility studies, it was established that pyrimethamine (as a single component) displayed high solubility at lower pH ranges (pH 1.2, 4.5) and lower solubility in pH 6.8 and water. Pyrimethamine was greatly affected by the presence of both artesunate and sulfadoxine. When pyrimethamine was investigated in combination with artesunate, the solubility trend was decreasing, as follows: ACE >> 0.1 N HCl >> NaCl >> water >> SBS >> PBS. However, compared to the single component of pyrimethamine only in water, PBS and SBS the solubility improved in the combination, particularly in water it improved substantially ($\pm$ 46-fold). A similar trend was observed for the combination with sulfadoxine, where pyrimethamine in the presence of sulfadoxine had an 8-fold increase in solubility in water, which was the highest increase, although the solubility is still low compared to other media.

Sulfadoxine proved stable in all media tested for both the single component and in combination. Moreover, in the presence of pyrimethamine, the solubility only improved in the ACE buffer solution and in water. For sulfadoxine in combination with pyrimethamine, the solubility trend decreases as follows: PBS >> SBS >> NaCl >> 0.1 N HCl >> water >> ACE.

Compatibility studies were employed using thermal activity microcalorimetry (TAM) for each API, individually and in combination, revealing no observable interactions. Notably, the study was carried out at 40°C. Additionally, differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) spectroscopy provided valuable insights into the properties of the APIs. During the FT-IR analysis, it seemed as if artesunate completely dispersed into pyrimethamine. This observation was corroborated by the DSC thermogram, where the melting point of the artesunate was absent and only the decomposition product was visible.

The outcomes of this research contribute significantly to our understanding of the interactions among artesunate, sulfadoxine, and pyrimethamine, shedding light on their solubility patterns and compatibility. Such insights are crucial for optimising the use of these APIs in combination

therapy for the effective prevention and treatment of *P. falciparum* malaria. Moving forward, the study underscores the importance of continued research in refining treatment strategies and addressing the evolving challenges posed by malaria, ensuring that our efforts remain at the forefront of combating this global health concern. Section 5.2 compiles further possible prospects to sustain momentum in advancing knowledge and strategies in the fight against malaria.

5.2 Future recommendations

- Investigate the reciprocal impact on solubility and compatibility when combining artesunate and sulfadoxine, as well as the trio of artesunate, sulfadoxine and pyrimethamine for possible interactions.
- Investigate the permeability of the FDC and the influence that the APIs exert on the permeability of the others, providing insights into their combined effects on drug delivery.
- Investigate the artesunate stability and explore innovative techniques to improve the stability, contributing to the development of more robust and durable pharmaceutical formulations.
- Investigate the HPLC methods to identify and characterise degradation products that might co-elute with artesunate, ensuring more accurate and reliable results.
- Develop an advanced HPLC method capable of simultaneously analysing artesunate, pyrimethamine, and sulfadoxine to offer a comprehensive and efficient analytical approach.

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ANNEXURE A: SPECIFICITY ON ARTESUNATE METHOD

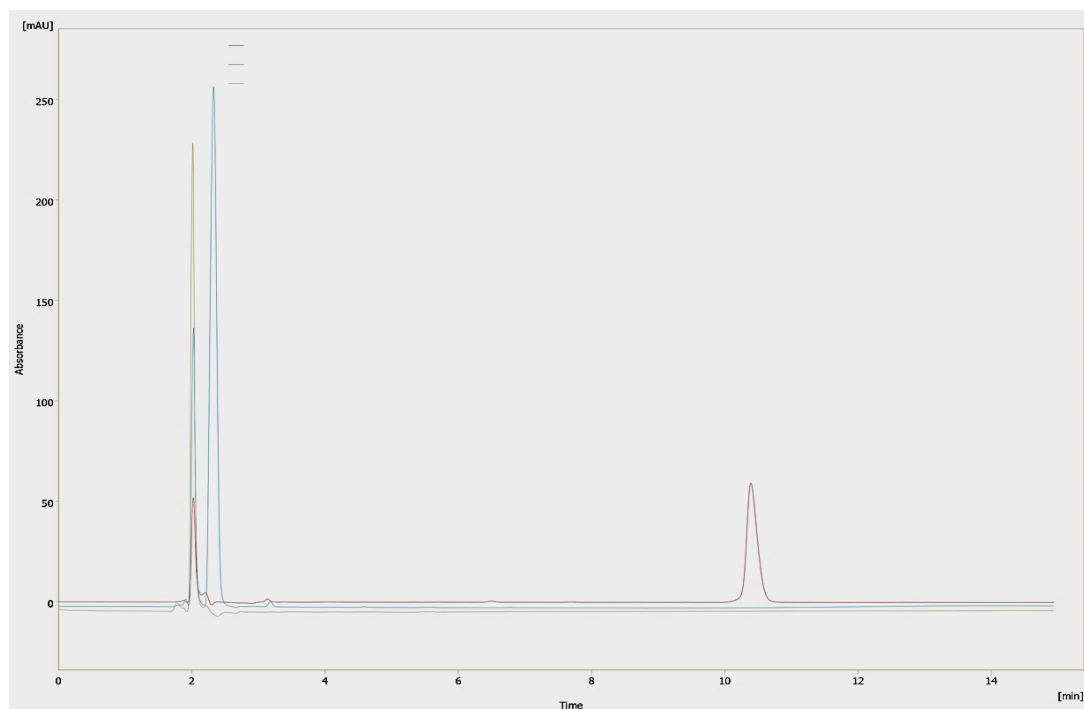


Figure A.1: Chromatographic overlay of artesunate standard (red) in 0.1 N HCl, pyrimethamine standard (blue) in 0.1 N HCl, and a blank injection of 0.1 N HCl solution (yellow).

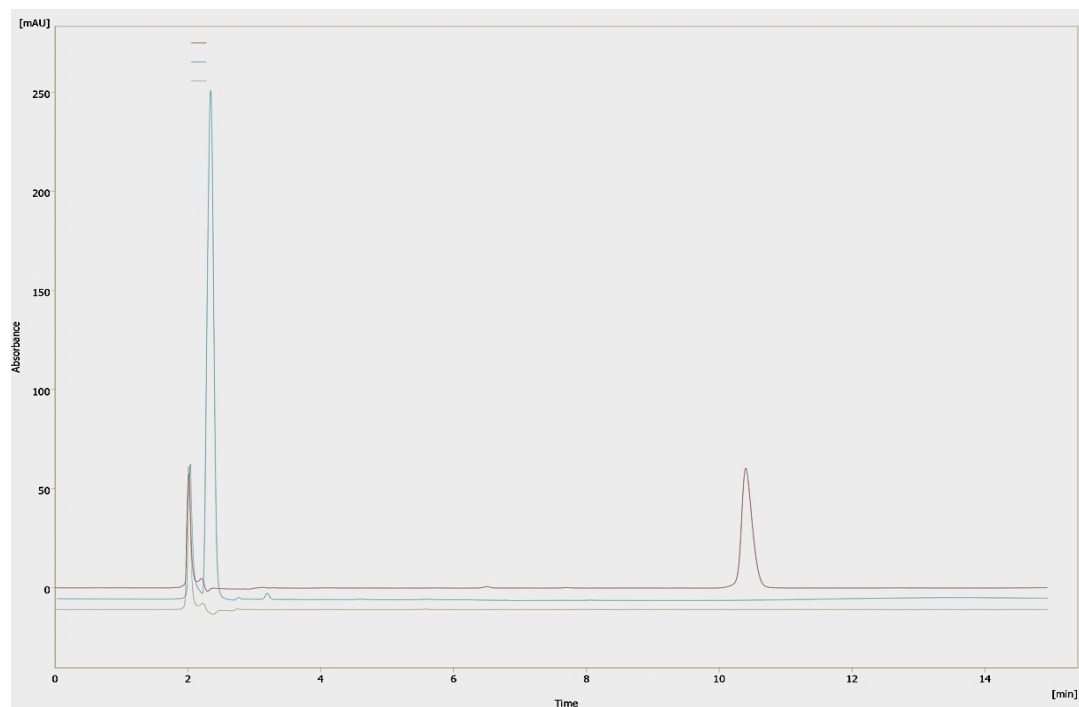


Figure A.2: Chromatographic overlay of artesunate standard (red) in NaCl, pyrimethamine standard (blue) in NaCl, and a blank injection of NaCl buffer solution (yellow).

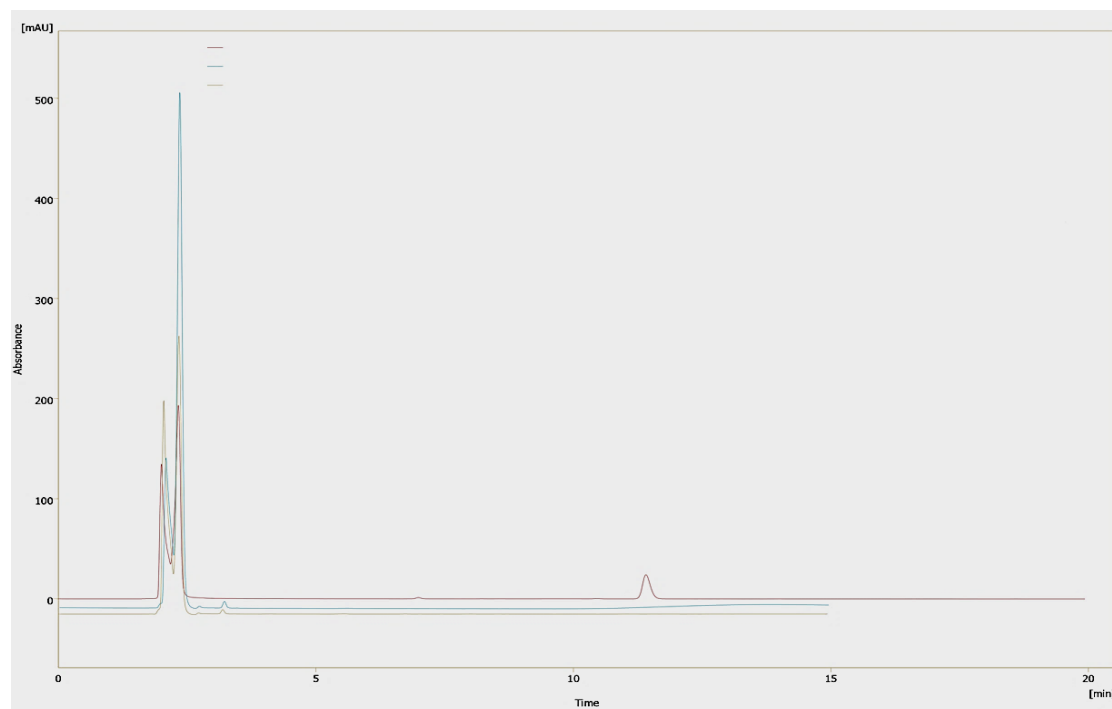


Figure A.3: Chromatographic overlay of artesunate standard (red) in ACE, pyrimethamine standard (blue) in ACE, and a blank injection of ACE buffer solution (yellow).

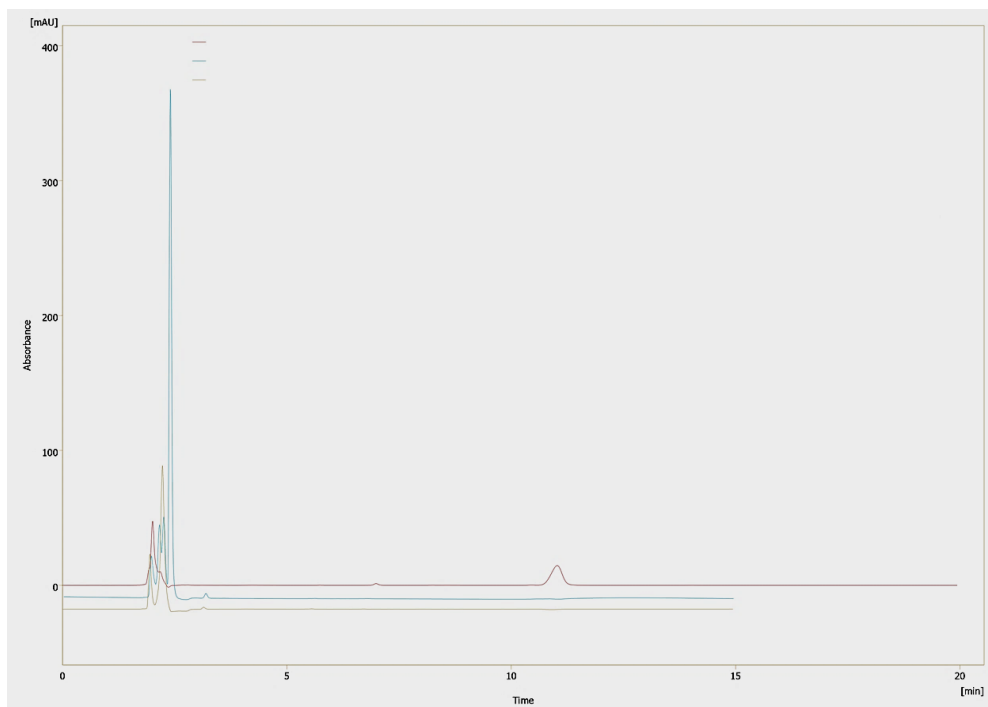


Figure A.4: Chromatographic overlay of artesunate standard (red) in PBS, pyrimethamine standard (blue) in PBS, and a blank injection of PBS buffer solution (yellow).

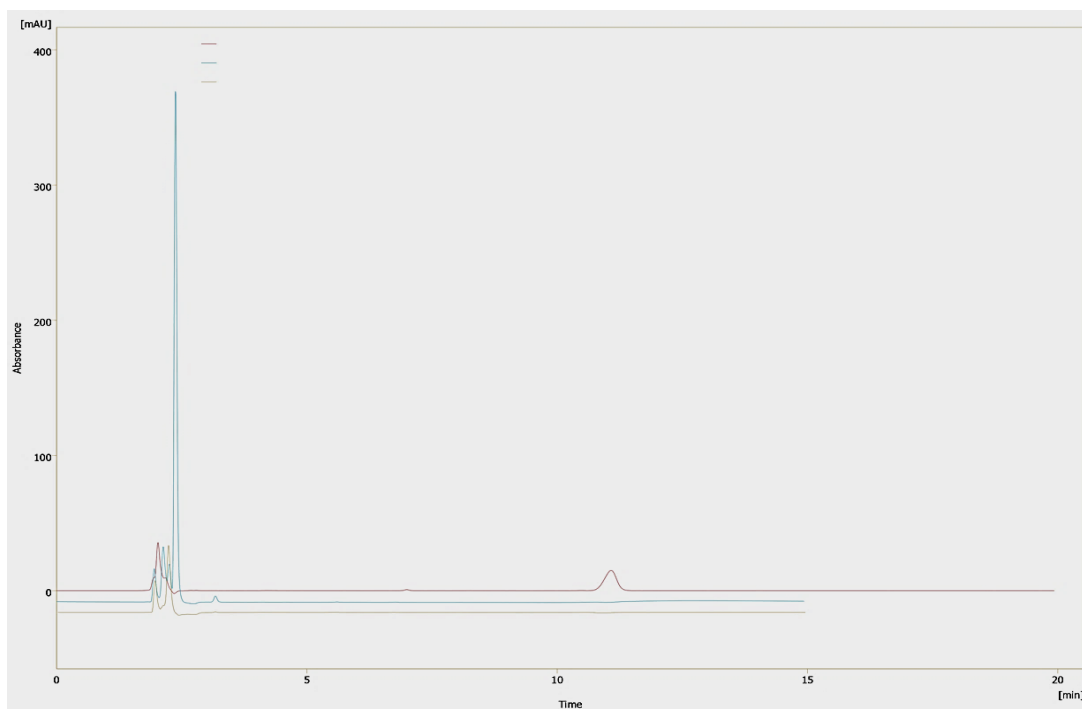


Figure A.5: Chromatographic overlay of artesunate standard (red) in SBS, pyrimethamine standard (blue) in SBS, and a blank injection of SBS buffer solution (yellow).

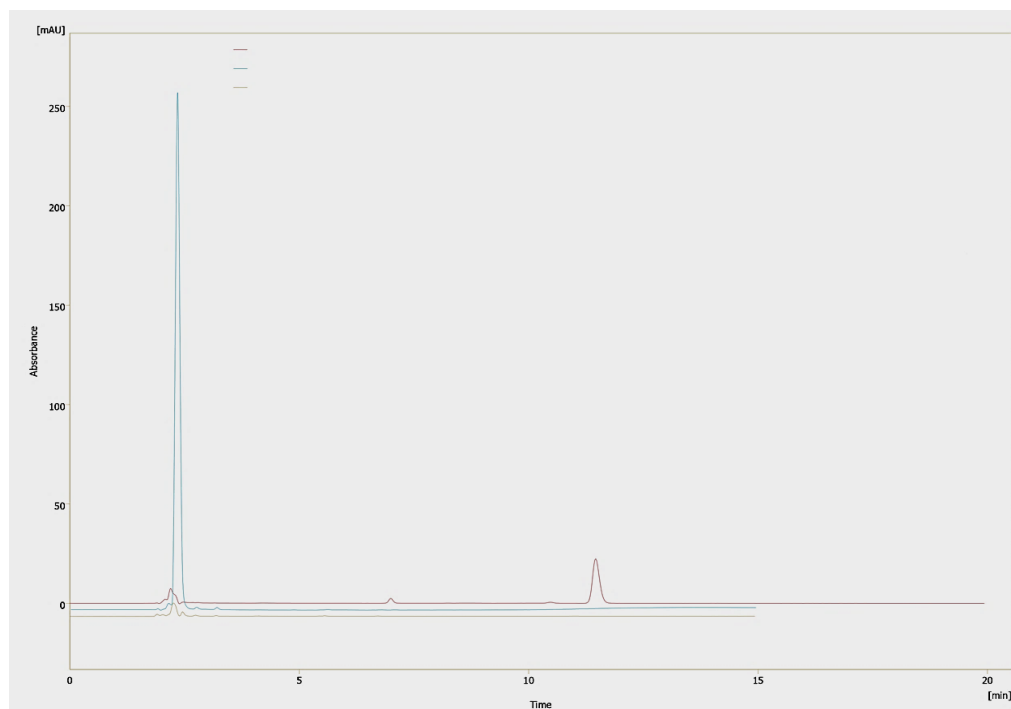


Figure A.6: Chromatographic overlay of artesunate standard (red) in water, pyrimethamine standard (blue) in water, and a blank injection of water (yellow).

ANNEXURE B: SPECIFICITY ON SULFADOXINE/PYRIMETHAMINE METHOD

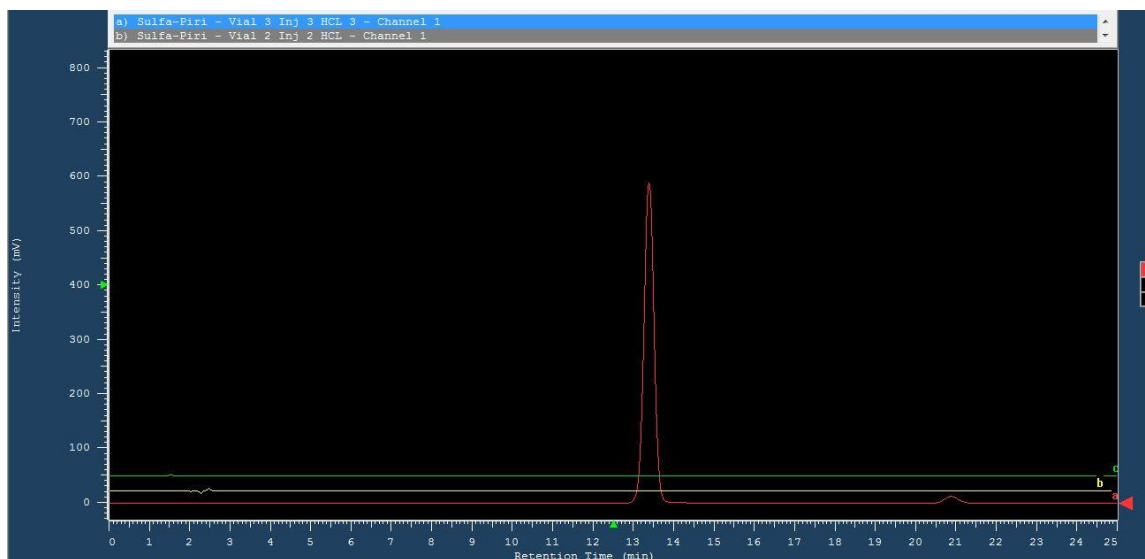


Figure B.1: Chromatographic overlay of pyrimethamine/sulfadoxine standard (red) in 0.1 N HCl, artesunate standard (green) in 0.1 N HCl, and a blank injection of 0.1 N HCl solution (yellow).

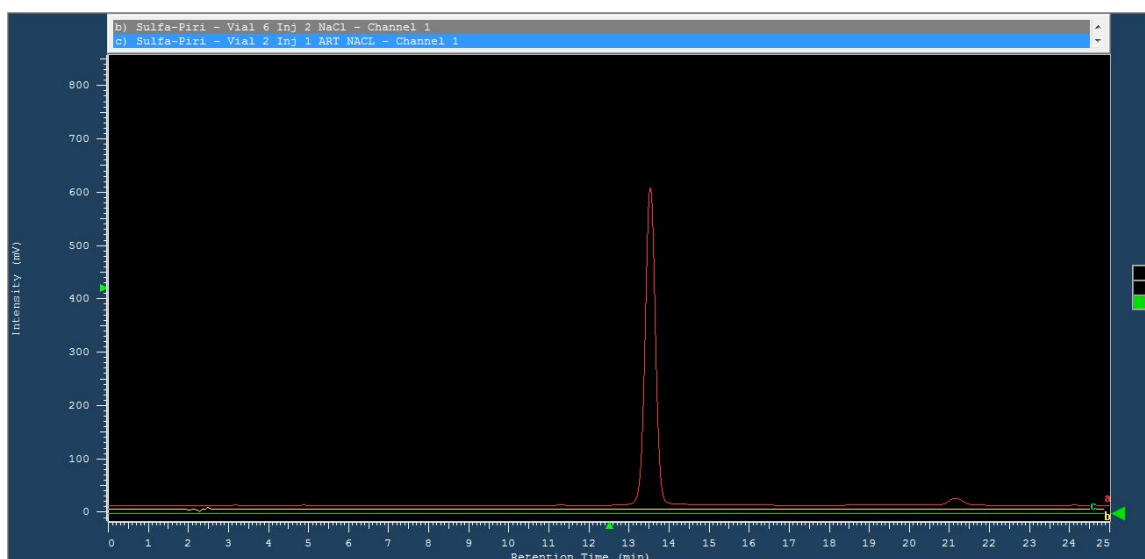


Figure B.2: Chromatographic overlay of pyrimethamine/sulfadoxine standard (red) in NaCl, artesunate standard (green) in NaCl, and a blank injection of NaCl buffer solution (yellow).

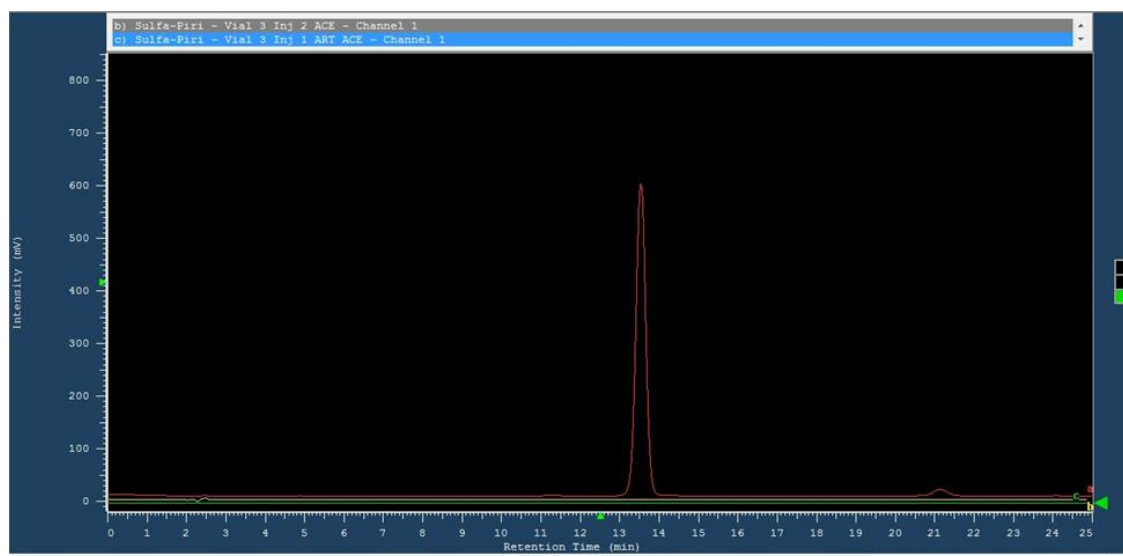


Figure B.3: Chromatographic overlay of pyrimethamine/sulfadoxine standard (red) in ACE, artesunate standard (green) in ACE, and a blank injection of ACE buffer solution (yellow).

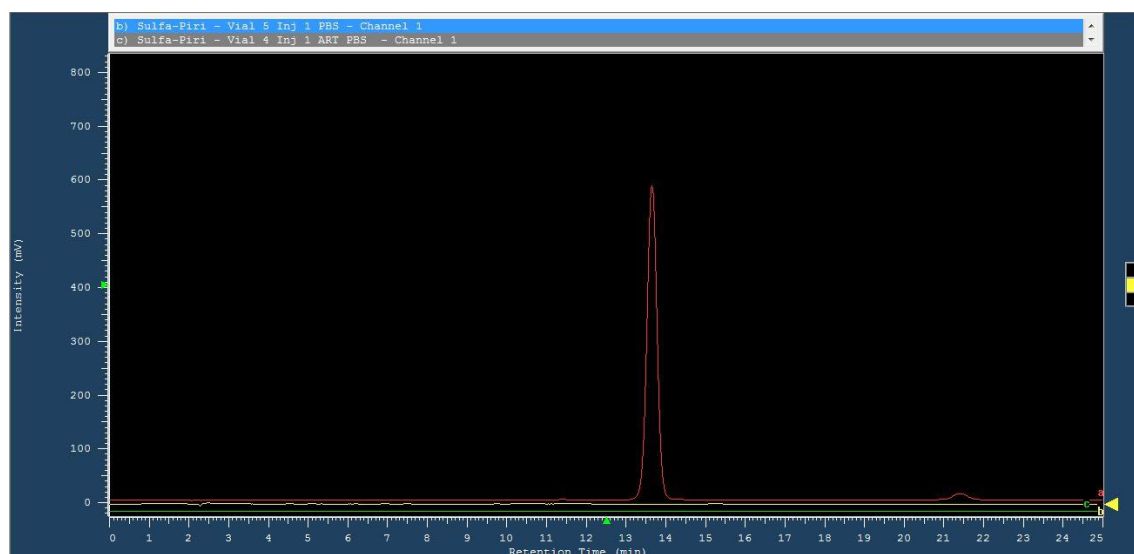


Figure B.4: Chromatographic overlay of pyrimethamine/sulfadoxine standard (red) in PBS, artesunate standard (green) in PBS, and a blank injection of PBS buffer solution (yellow).

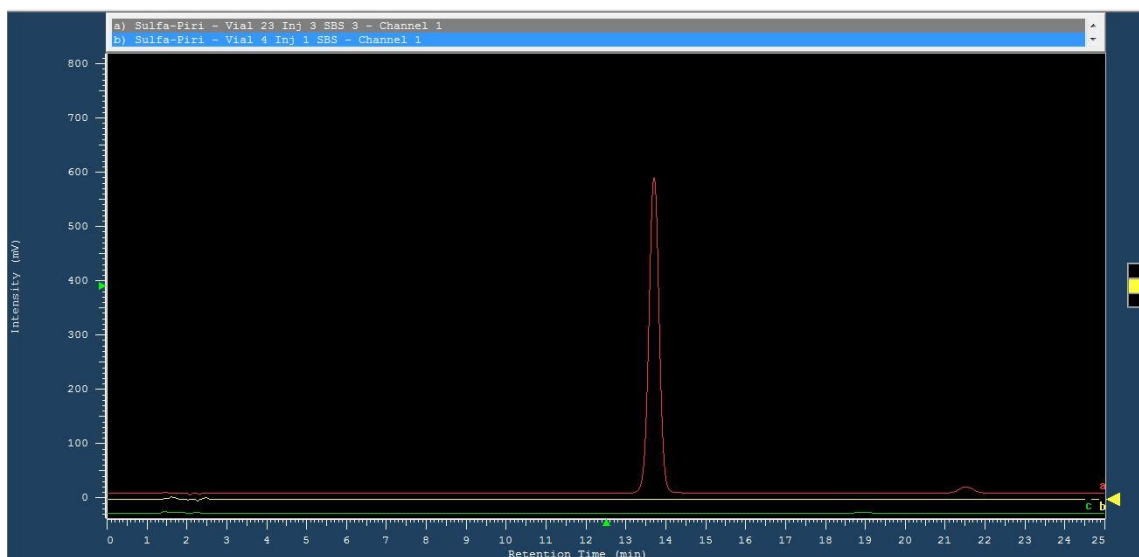


Figure B.5: Chromatographic overlay of pyrimethamine/sulfadoxine standard (red) in SBS, artesunate standard (green) in SBS, and a blank injection of SBS buffer solution (yellow).

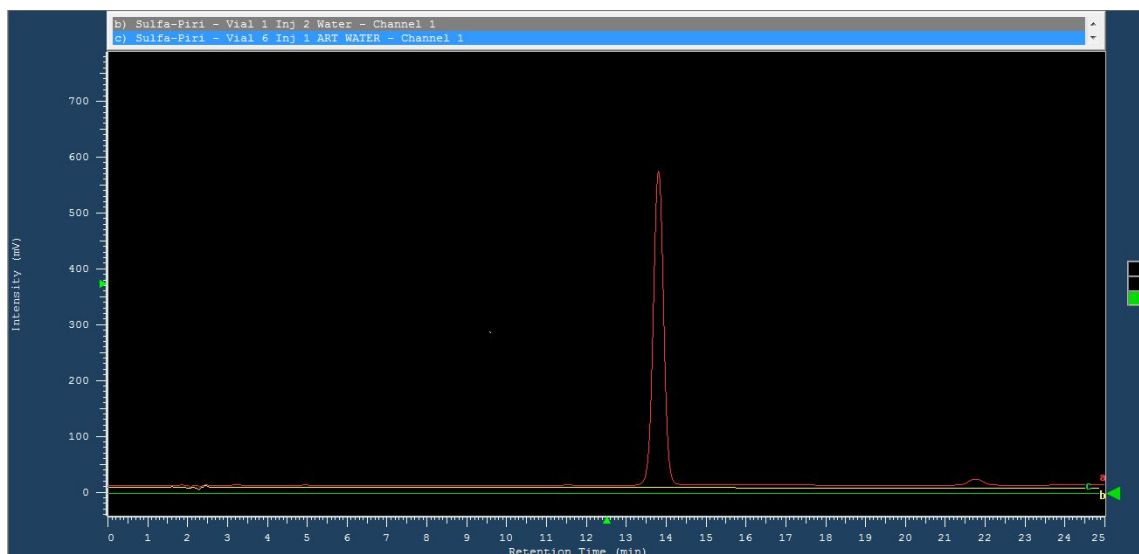


Figure B.6: Chromatographic overlay of pyrimethamine/sulfadoxine standard (red) in water, artesunate standard (green) in water, and a blank injection of water (yellow).