Quality specifications for the antimalarial compound lumefantrine and products thereof

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TABLE OF CONTENTS

Aims and Objectives

Malaria is currently one of the biggest concerns to the World Health Organization (WHO), as it is daily causing the deaths of vast numbers of people, especially in Africa. A lack of education, resources, infrastructure and the prevalence of counterfeit, or substandard medicines in the marketplace, have become some of the main reasons for the growing numbers of malaria related mortalities and resistance to antimalarial treatments.

The fixed-dose-combination (FDC) of artemether/lumefantrine is currently recommended as part of the WHO's regime for the treatment of malaria (WHO, 2006). Since a monograph for only the artemether active pharmaceutical ingredient (API) already exists (International Pharmacopoeia (Ph.Int., 2008)), it has become a high priority to the WHO that monographs for the lumefantrine API and for the FDC forms of these two actives be developed, in order to protect the effectiveness of these products.

Furthermore, both artemether and lumefantrine are poorly soluble in aqueous solutions (Ph.Int. (2008); per manufacturer information provided by WHO). Their classification by the biopharmaceutics classification system (BCS) is thus IV/II (Lindenburg *et al.*, 2004:70). Hence, for APIs, where the absorption is limited, due to its poor dissolution (solubility), differences in polymorphic forms are likely to affect the bioavailability of the final product. No literature with regards to the polymorphic behaviour of artemether and lumefantrine was, however, available at the time of this study.

The following study objectives were therefore set and pursued:

- Conduct a polymorphic screening of the artemether and lumefantrine APIs and the characterisation of their recrystallisation products, using a representative set of analytical techniques (Chapter 3);
- Develop suitable methods for the quality control testing of the lumefantrine API for possible inclusion in a monograph, according to the requirements of the Ph.Int. (Chapter 4);
- Validate applicable methods in the lumefantrine API monograph, according to international standards (Chapter 4);
- Develop suitable methods for quality control testing of artemether/lumefantrine combination tablets and oral suspensions, for possible inclusion in the proposed monograph and in the Ph.Int. (Chapter 5);

- Validate applicable methods in the artemether/lumefantrine tablets and oral suspensions monograph, according to international standards (Chapter 5);
- Evaluate the transferability of the developed methods, in order to evaluate the potential robustness thereof (Chapter 6); and
- Perform a quality survey, using the newly developed monographs, to evaluate the quality of selected artemether/lumefantrine anti-malarial medicines in selected Sub-Saharan African countries (Chapter 6).

The main contributions that these studies would make to the pharmaceutical industry would include the following:

- Adoption of the proposed monographs for inclusion into the Ph.Int. for use by manufacturers and independent quality control and quality assurance laboratories.
- The outcomes of the polymorphic screening studies were included, because of the value in providing the pharmaceutical industry, especially manufacturers of these APIs and products, with potentially important information relating to the performance of products containing these APIs.
- The monograph developed for lumefantrine API would be used by the pharmaceutical industry in determining and ensuring the quality of lumefantrine raw materials, before their release onto the market.
- The FDC monographs developed would be used in the development of artemether/lumefantrine dosage forms of acceptable quality and the evaluation of dossiers for these products.

Finally, the adoption of the proposed new monographs for inclusion into the Ph.Int. would assist the WHO in its combat against the distribution of counterfeit and inferior antimalarials. This would assist in reducing resistance to these illegal treatments through early detection, and thus in the numbers of malaria related deaths. It is anticipated that all these would ultimately contribute to improving the quality of lives and communities.

ABSTRACT

Malaria has been responsible for the deaths of thousands of people annually and is considered one of the biggest health challenges globally. In order to ensure that patients in malaria affected areas receive products of suitable quality, it is important that effective pharmacopoeial monographs for these medicines are available for distinguishing between good and inferior quality products, prior to distribution to the public.

With artemether and lumefantrine forming part of the current, most effective treatment regimes against malaria, the development of monographs for the lumefantrine active pharmaceutical ingredient (API) (a monograph already exists for the artemether API) and for artemether/lumefantrine combination dosage forms, has become a high priority to the World Health Organization (WHO) for inclusion in the International Pharmacopoeia (Ph.Int.).

During this study, both APIs were also evaluated for the possible occurrence of polymorphism, in order to provide manufacturers with additional information regarding identified polymorphic forms.

During the polymorphic screening of artemether, the physico-chemical properties of the different recrystallisation products demonstrated results similar to those of the raw materials tested. Although the polymorphic screening of lumefantrine revealed no new polymorphic forms, it was found that the X-ray powder diffraction (XRPD) patterns were influenced by preferred orientation and that the variation in melting points obtained, correlated well with differences observed in particle size and/or morphology. It therefore illustrated the importance for manufacturers of lumefantrine to consider the possibility of the melting point being influenced by crystal habit and particle size, and would it be advisable not to characterise and identify lumefantrine, based on the melting point alone.

The physico-chemical analytical results obtained during the polymorphic screening of artemether and lumefantrine furthermore revealed that only one crystal packing for each API exists.

Methods for the two new monographs were developed in accordance with WHO requirements and were validated compliant with the International Conference on Harmonisation (ICH) guidelines on the validation of methods.

The methods developed for lumefantrine during this study, included the following: The chosen identification tests comprised a standalone infrared method, or as an

alternative a combination of ultraviolet spectrophotometry (UV-VIS) and thin layer chromatography (TLC) methods. The standard pharmacopoeial methods for heavy metals, sulfated ash (residue on ignition) and loss on drying were proposed. For the determination of the related substances (impurities from synthesis and degradation products), a high performance liquid chromatography (HPLC) method and a newly developed TLC method were included, with the monograph allowing a choice.

Furthermore, a titration with perchloric acid was decided upon for the assay of the lumefantrine API. An alternative UV spectrophotometric assay method (not included in the Ph.Int. monograph) was developed using a UV spectrophotometric method where the $A_{1cm}^{1\%}$ value in methanol was established as 331.4.

For the monographs of artemether/lumefantrine tablets and powder for oral suspension two identification tests were developed for use in combination, namely TLC and HPLC methods.

A TLC limit test was developed for the related substances of artemether, which proved to be less stable than lumefantrine. The developed method made the simultaneous assaying of artemether and lumefantrine, using HPLC, possible.

The proud outcome of this research project was the adoption of the final proposed monographs for the lumefantrine API and artemether/lumefantrine combination dosage forms, during the 42nd meeting of the WHO's Expert Committee on Specifications for Pharmaceutical Preparations (July 2008, Geneva), for inclusion in the Ph.Int.

The methods for identification, assay and related substances of the artemether/lumefantrine tablets monograph were verified by an independent surveillance study, during which the results demonstrated that the methods were easily transferable between quality control (QC) laboratories. During this study, the proposed monograph was used to test 108 batches of artemether/lumefantrine tablets, sourced by the WHO. The generated results were able to identify batches of substandard quality and thus proved the monograph methods to be suitable for distinguishing between suitable or inferior quality, and even counterfeit batches.

The conclusion that could be drawn from the outcomes of this study was that the use of the methods in these monographs during quality control testing, should indeed result in a reduction in substandard and/or counterfeit products being distributed to the public, thus in reducing the number of malaria related deaths and the escalating occurrence of resistance against antimalarial treatment regimes.

UITTREKSEL

Malaria veroorsaak jaarliks die dood van duisende mense en word as een van die grootste gesondheidsuitdagings ter wêreld beskou. Om te verseker dat pasiënte in malaria geaffekteerde gebiede goeie kwaliteit medisyne ontvang, is dit belangrik dat effektiewe farmaseutiese monograwe vir hierdie produkte beskikbaar is, ten einde tussen medisyne van goeie en swak gehalte te kan onderskei, alvorens verspreiding na die publiek.

Met artemeter en lumefantrien tans deel van die mees effektiewe behandelings teen malaria, het die ontwikkeling van monograwe vir die lumefantrien aktiewe bestanddeel (’n monograaf bestaan alreeds vir die artemeter aktief) en vir artemeter/lumefantrien kombinasieprodukte, hoog op die Wêreldgesondheidsorganisasie (WGO) se prioriteitslys beland, vir insluiting in die Internasionale Farmakopie (International Pharmacopoeia (Ph.Int.)).

Tydens hierdie studie is beide die artemeter en lumefantrien aktiewes ook vir die moontlike voorkoms van polimorfisme evalueer, ten einde vervaardigers van bykomende inligting aangaande enige geïdentifiseerde polimorfiese vorme te voorsien.

Tydens die polimorfiese siftingsproses van artemeter, het al die verskillende herkristallisasie-produkte soortgelyke fisies-chemiese eienskappe, in ooreenstemming met dié van die grondstofmonsters wat getoets is, getoon. Afgesien daarvan dat die polimorfiese sifting van lumefantrien geen nuwe polimorfe vorms opgelewer het nie, is bevind dat die X-straalpoeierdiffraksiepatrone deur voorkeuroriëntasie beïnvloed is en dat die variasie in smeltpunte sterk met waargenome verskille in grootte en/of morfologie van die deeltjies ooreengestem het. Hierdie uitkomste het die belang daarvan, dat vervaardigers van lumefantrien die moontlikheid dat die smeltpunt deur die kristalgewoonte en deeltjiegrootte beïnvloed kan word, in gedagte moet hou, beklemtoon. Dit sou gevolglik ongewens wees om lumefantrien op grond van smeltpunt alleen te identifiseer.

Die fisies-chemiese resultate, tydens die polimorfiese sifting van artemeter en lumefantrien verkry, het voorts getoon dat slegs een kristalpakking vir elk van die aktiewe bestanddele bestaan het.

Metodes vir die twee nuwe monograwe is in ooreenstemming met die WGO se vereistes ontwikkel en is volgens die riglyne van die Internasionale Konferensie vir Harmonisering (ICH) vir die validasie van metodes valideer.

Die volgende metodes vir lumefantrien is tydens hierdie studie ontwikkel vir insluiting in die spesifieke monograaf: Die geselekteerde identifikasietoets was 'n alleenstaande, infrarooi metode, met as alternatief 'n kombinasie van 'n ultravioletspektrofotometriese en 'n dunlaagchromato-grafiese metode. Die standaardtoetse, soos deur die Ph.Int. beskryf, is vir swaar metale, residu na verbranding en verlies tydens droging voorgestel en aanvaar. Vir die bepaling van verwante produkte (sintese-onsuiwerhede en afbraakprodukte), is 'n hoëdrukvloeistofchromatografiese (HPLC) metode en 'n nuut-ontwikkelde dunlaagchromatografiese toets ingesluit, terwyl die monograaf 'n keuse tussen enige van hierdie twee toetse toegelaat het.

Daar is voorts op 'n titrasie met perchloorsuur vir die inhoudsbepaling van die lumefantrien aktief besluit. 'n Alternatiewe ultravioletspektrofotometriese analise (wat nie in die monograaf ingesluit is nie) en waarin gebruik, waarvan die waarde in metanol as 331.4 bepaal is.

Vir die monograwe van artemeter/lumefantrien in kombinasieprodukte (tablette en poeier vir suspensie), is twee identifikasietoetse ontwikkel vir gebruik in kombinasie, naamlik 'n dunlaagchromatografiese en 'n HPLC-metode.

'n Dunlaagchromatografiese limiettoets is vir die afbraakprodukte (onsuiwerhede) van artemeter ontwikkel, wat getoon het minder stabiel as lumefantrien te wees. Die ontwikkelde metode het die gelyktydige inhoudsbepaling van artemeter en lumefantrien op HPLC moontlik gemaak.

Die trotse uitkomste van hierdie navorsingsprojek was die aanvaarding van die finaal-voorgestelde monograwe vir die lumefantrien aktiewe bestanddeel en vir artemeter/lumefantrien in kombinasie-dosisvorme, tydens die 42ste vergadering van die WGO se "Expert Committee on Specifications for Pharmaceutical Preparations" (Julie 2008, Genève), vir publikasie in die Ph.Int.

Die metodes vir identifikasie, inhoudsbepaling en die bepaling van artemeter afbraakprodukte in die monograaf vir artemeter/lumefantrien-tablette, is tydens 'n onafhanklike marksteekproef geverifieer, waartydens die uitslae wel getoon het dat die metodes geredelik tussen verskillende gehaltebeheerlaboratoriums oordraagbaar is. Tydens hierdie studie is die voorgestelde monograwe vir die toetsing van 108 lotte van

artemeter/lumefantrien-tablette, soos deur die WGO verskaf, gebruik. Die resultate wat ingesamel is, kon substandaard lotte identifiseer en het dus bewys gelewer dat die metodes in die monograwe geskik was om tussen produkte van goeie en swak kwaliteit en selfs vervalsde produkte onderskeid te tref.

Die gevolgtrekking wat uit hierdie studie gemaak kon word is dat die gebruik van die metodes in die voorgestelde monograwe, tydens kwaliteitskontrole-toetsing, inderdaad tot die identifisering en vermindering van substandaard en/of vervalsde produkte, wat huidig na die publiek versprei word, aanleiding behoort te gee. Dit behoort dus daarin te slaag om ‘n afname in die aantal malaria-verwante sterftes en die toenemende voorkoms van weerstand teen swak kwaliteit medikasie, te weeg te bring.

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CHAPTER 1

Malaria - A global threat

1.1 Introduction

Malaria is one of the biggest threats to global health today. In order to support the challenges of product development and clinical testing of urgently needed medicines, the Special Programme for Research and Training in Tropical Diseases (TDR), sponsored by United Nations International Children's Emergency Fund (UNICEF), United Nations Development Programme (UNDP), the World Bank and the World Health Organization (WHO) initiated programs, such as the Medicines for Malaria Venture (MMV) joined hands with other partners, such as the Drugs for Neglected Diseases Initiative (DNDi) (TDR News, 2007:13).

In November 2008, the Global Malaria Action Plan (GMAP) launched a new malaria research initiative. The Malaria Eradication Research Agenda (MaIERA) followed upon the Roll Back Malaria Partnership's (RBM) announcement of its new efforts to eliminate and eventually eradicate malaria globally. The TDR assists through TropIKA.net and the initiative is supported by die Bill and Melinda Gates Foundation, among others (TDR News, 2008:9).

This research would cost at least US $750 million annually over the next ten years. The goals include up scaled access to bed-nets, indoor spraying, early and effective diagnosis and treatment, preventive treatment for pregnant women in Africa by 2010, reducing the number of deaths to near zero by 2015, with the long term goals being to maintain a near zero death rate and through research, to eventually find a way to eradicate malaria completely (TDR News, 2008:9).

According to Maurray M. Lumpkin, M.D. and deputy commissioner for the International and Special Programs of the United States (US) Food and Drug Administration (FDA), malaria is a global, life threatening disease and is it encouraging to have new treatments available, especially for children. The FDA has currently approved Coartem® tablets (artemether and lumefantrine) for the treatment of acute and uncomplicated malaria in children (weighing more than 5 kg) and in adults (US FDA, 2009a).

This chapter will focus on the global threat of malaria, the disease itself, its treatment and the problems associated with malaria treatment, such as resistance, substandard and counterfeit/adulterated products.

1.2 Malaria - a bigger problem than expected

About 3.3 billion people (half the world's population) are at risk of malaria, particularly those living in lower income countries (WHO, 2009a; CDC, 2007).

- 250 million people contract malaria annually (WHO, 2009a).
- About 880 000 people die from malaria each year (WHO, 2009a).
- Every 30 seconds a child in Africa dies from malaria (WHO, 2009a).
- 90% of all malaria induced deaths occur in sub-Saharan Africa (IMC, 2007).
- Malaria is responsible for a 1.3% economical growth penalty per year in some African countries (IMC, 2007).
- Malaria costs Africa more than $12 billion in lost gross domestic product (GDP) every year (IMC, 2007).
- Pregnant women are at high risk, not only of dying from the complications of severe malaria, but also spontaneous abortion, premature delivery, or still birth (WHO, 2009a).
- Malaria causes severe maternal anaemia and is responsible for about one third of premature, low weight babies (RBM, 2009).
- Malaria contributes to the deaths of an estimated 10 000 pregnant women and up to 200 000 infants each year, just in Africa (WHO, 2009a).
- One in five of all childhood deaths in Africa is caused by malaria. An African child will on average have between 1,6 and 5,4 episodes of malaria fever each year (WHO, 2009a).
- Malaria may induce epilepsy and learning difficulties (RBM, 2009).
- Malaria is both preventable and treatable (RBM, 2009).

The most important tools for the prevention and treatment of malaria include:

- Insecticides and insecticide treated nets (Table 1.2)
- Intermittent preventive treatment in pregnancy.
- Antimalarial drug combination therapy (RBM, 2009).

1.3 Malaria - a tropical disease

1.3.1 Pathogenesis of malaria

The life cycle of the malaria parasite is illustrated in Figure 1.1. An infected female mosquito (*Anopheles*) will bite a person (host) for the human blood she feeds on. The young protozoan *Plasmodium* parasites (any of the four species, i.e. *falciparum, vivax, malariae* and *ovale*, of which *falciparum* is the most life threatening) will enter into the host's blood, *via* the insect saliva. The young parasites (sporozoites) are transported in the blood to the hepatocytes where they penetrate the liver and grow into merozoites (schizogony). After an incubation period of about 9 days, the merozoites migrate from the liver (as gametocytes) and penetrate the erythrocyte. They rapidly multiply through a series of schizogonous cycles. In the erythrocytes, they become ameboid trophozoites that feed on hemoglobin and produce hemozoin, a dark insoluble pigment, which accumulates in the host cells. When the erythrocyte bursts, releasing the next generation of merozoites, this metabolic product is also released. The trophozoites continue to undergo schizogony in the erythrocyte and produce merozoites, which continue to invade new erythrocytes, multiply and progressively break the cells down. When the parasites' metabolic products (hemozin) are released into the patient's circulation, it results in the symptoms of malaria, such as chills and fever (Hickman *et al.,* 1997:229; Miller *et al*., 1994:1878).

When an uninfected female mosquito bites an infected host, the gametocytes will be taken in with the blood, and the mosquito will be infected. After the parasites undergo several stages of growth in the mosquito, she will too be spreading the disease (Hickman *et al.,* 1997:229).

Some of the merozoites produce microgametocytes and macrogametocytes after a few cycles of schizogony in the erythrocytes. When a feeding mosquito (*Anopheles*) ingests these, they mature into gametes. Fertilisation occurs and the zygote becomes a motile ookinite, which penetrates the mosquito's intestinal wall and becomes an oocyst. Sporogony occurs within the oocyst and produces thousands of sporozoites. When the oocyst ruptures, the sporozoites migrate to the salivary glands of the mosquito, from where they penetrate the human body upon feeding. The development phase in the mosquito requires 7 - 18 days in favourable weather (Hickman *et al.,* 1997:229; Miller *et al*., 1994:1879).

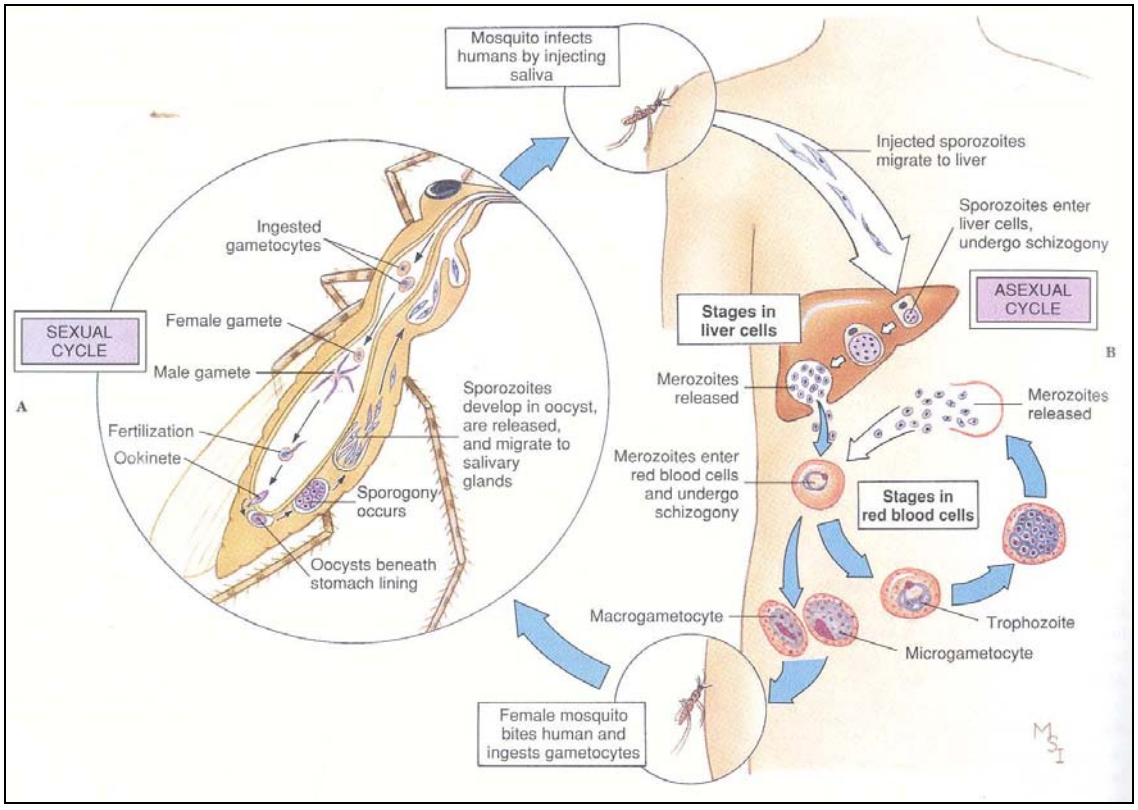


Figure 1.1 The life cycle of the malaria parasite (Hickman *et al.,* 1997:230).

1.3.2 Signs and symptoms of malaria

The typical symptoms of uncomplicated malaria will occur within 10 - 15 days after a person is infected, and are mostly non-specific and very similar to systemic viral infections. It presents itself with regular intervals of fever spikes (the hot stages, up to 41ºC, due to the invasion of the erythrocytes), chills and rigors (the cold stage, when the erythrocytes burst), as well as diaphoresis (the sweating stage). Nausea, vomiting, fatigue, diarrhea, headaches, joint and body pains, anaemia and even jaundice, are common symptoms of uncomplicated malaria, which disappear when the parasites are killed (Tierney *et al*., 2007:1518; Directors of Health Promotion and Education, 2007).

When malaria is caused by *Plasmodium falciparum* and not treated effectively in the early stage, it may lead to severe malaria within a few hours. Severe malaria is almost always fatal and presents a variety of life threatening complications, like hepatic and renal failure, respiratory distress syndrome and cerebral malaria (when the infected erythrocytes obstruct the cerebral vessels), to mention only a few (WHO, 2006:5-6).

1.3.3 Diagnosis of malaria

According to the current medical diagnosis and treatment (CMDT) (Tierney *et al*., 2007:1517), the diagnosis of malaria includes the following:

- History of exposure to a malaria-endemic area(s).
- Periodic attacks of sequential chills, fever and sweating.
- Headaches, malaise, myalgia, nausea, vomiting, splenomegaly, anaemia and leucopenia.
- Characteristic parasites in erythrocytes, identified in thick or thin blood films.
- Complications of *falciparum* malaria, i.e. cerebral findings (mental disturbances, neurological signs, convulsions, coma), enteric or cholera-like stools, dark urine and anuria.

One of the objectives in the effective disease management of malaria and the reduction in the unnecessary use of antimalarials, is the timely and accurate diagnosis of malaria. In young children, who are the most vulnerable, the high sensitivity of malaria diagnosis is extremely important, since severe malaria can be fatal in an instant. Malaria diagnosis is based on a clinical and parasitological (or confirmatory) diagnosis (WHO, 2006:5, 8-9).

1.3.3.1 Clinical diagnosis

The recommendations of the WHO Expert Committee on Malaria are used for clinical diagnosis (WHO, 2000:18). They state:

- The signs and symptoms of malaria are mostly non-specific, therefore clinical diagnosis is generally based on fever, or a history thereof.
- Generally, in settings where the risk of malaria is low, clinical diagnosis of uncomplicated malaria is based on the degree of exposure to malaria and a history of fever in the previous 3 days, with no features of other severe diseases.
- In settings, where the risk of malaria is high, clinical diagnosis should be based on a history of fever in the previous 24 hours and/or the presence of anaemia, for which paleness of the palms appears to be the most reliable sign in young children.

The WHO/UNICEF strategy for the Integrated Management of Childhood Illness (IMCI) has developed practical algorithms for the management of sick children having fever, and who come from rural malaria areas, where no facilities for laboratory diagnosis are available (IMCI, 1999).

1.3.3.2 Parasitological diagnosis

This diagnosis is mainly performed by means of two methods, i.e. light microscopy (when performed by well trained staff, this method is of low cost and high sensitivity and specificity) and rapid diagnostic tests (RDT) for the detection of the parasite antigen. RDT results, however, are variable, they are sensitive to high temperatures and humidity and not always cost-effective (especially in sub-tropical and tropical areas where malaria mostly occurs). If the parasitological diagnosis results are not available in less than 2 hours, the patient should be treated on the basis of clinical diagnosis (WHO, 2006:9).

Table 1.1 Summary of recommendations for parasitological diagnosis, reproduced from WHO (2006:11)

In areas of low to moderate transmission: Prompt parasitological confirmation of the diagnosis is recommended, before treatment is started. This should be achieved through microscopy, or, where not available, RDTs.
In areas of high stable malaria transmission: The prior probability of fever in a child being caused by malaria is high. Children under 5 years of age should therefore be treated on the basis of a clinical diagnosis of malaria. In older children and adults, including pregnant women, a parasitological diagnosis is recommended, before treatment is started.
In all suspected cases of severe malaria: A parasitological confirmation of the diagnosis of malaria is recommended. In the absence of, or a delay in obtaining parasitological diagnosis, patients should be treated for severe malaria on clinical grounds.

In order to control malaria, early diagnosis and prompt treatment after diagnosis are essential. Effective treatment can shorten the duration of the infection and thus prevent further complications, which usually lead to the great number of deaths (WHO, 2009a:8).

1.3.4 Geographical prevalence of malaria

Malaria parasites can only survive in areas where they can complete their life cycle in the *Anopheles* mosquitoes. These areas are normally characterised by high temperatures, humidity and rainfall, thus tropical and sub-tropical areas, as illustrated in Figure 1.2.

Transmission in such favourable areas will in some instances not occur, due to factors such as:

- High altitudes.
- Cooler seasons in some areas (more seasonal and less intense transmission).
- Deserts (excluding oases).

- Areas where transmission has been interrupted through successful eradication.
- Islands in the Pacific Ocean, where there are no local *Anopheles* species capable of transmitting malaria.
- *Plasmodium falciparum* cannot complete its growth cycle in the mosquito at temperatures below 20ºC, and will therefore not be transmitted (CDC, 2007).

The warmer regions closer to the equator will generally lead to more intense transmissions of *Plasmodium falciparum* all year round (CDC, 2007).

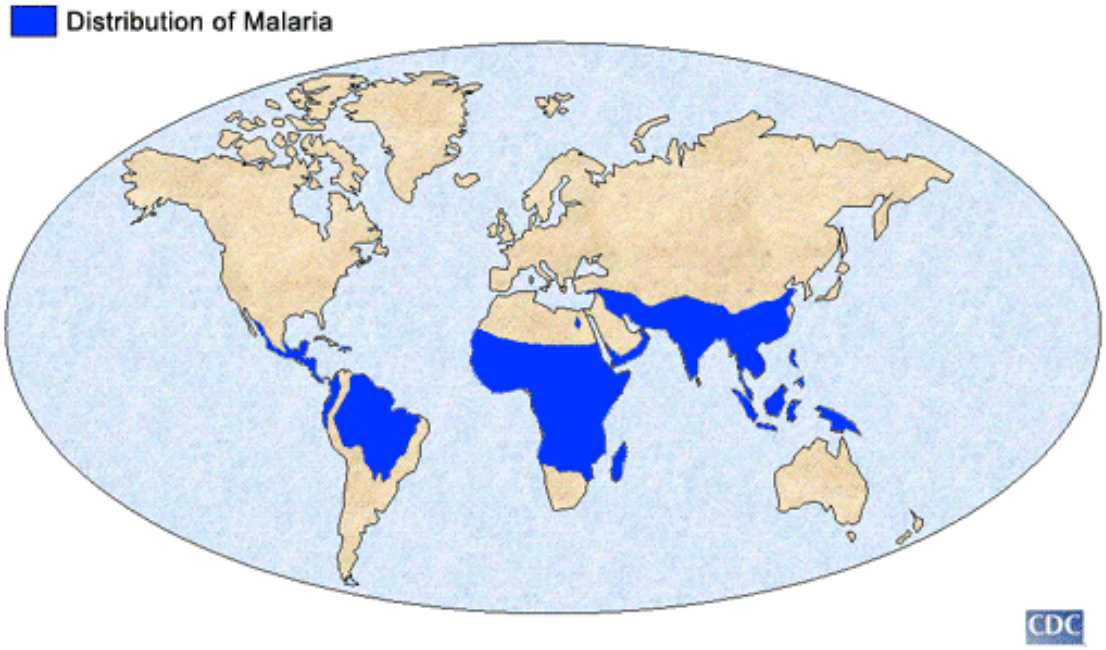


Figure 1.2 The geographical distribution of malaria (CDC, 2007).

1.3.5 Prevention from contracting malaria

In communities (mostly in sub-Saharan Africa and parts of Oceania), where the populations are constantly exposed to high inoculation rates (entomological inoculation rate (EIR) >10/year), people acquire partial immunity to the clinical disease from an early age. In these areas, it is usually the children under the age of five that are the highest at risk of severe malaria and death, if left untreated, due to their lack of immunity against these parasites (WHO, 2006:1).

According to the guidelines for the treatment of malaria (WHO, 2006:1), partial immunity to malaria is lost if a person leaves this area. In areas where malaria is unstable and the rates of EIR is usually <5/year, any form of immunity is retarded and therefore all age groups are at risk of contracting severe malaria, if effective treatment is unavailable.

Although a certain degree of partial immunity can be acquired, no vaccine is available against malaria. The best way to avoid contracting malaria is to avoid bites by the infected *Anopheles* mosquitoes. The Directors of Health Promotion and Education, Malaria (2007:1-4) and the Malaria Manual for Community and Health Workers (Department of Health, 2006:15-21) recommend the following to avoid mosquito bites:

- Avoid/reduce contact with mosquitoes at night-time (which is their feeding time) by wearing long-sleeved clothing and long pants, treated with insecticide, if possible.
- Use insect repellent on exposed skin.
- Stay in well-screened areas during the evening.
- Spray living and sleeping areas with an insecticide (Table 1.2).
- Use a bed-net (preferably treated with insecticide), especially when sleeping in a room that is not screened, or air-conditioned.
- Use suitable prophylaxis (different active pharmaceutical ingredients (APIs) are effective for specific areas).
- In case of the incidence of any malaria signs or symptoms, immediately seek medical help .

Although these measures may sound fairly easy for travelers and tourists, they often are not practical, nor possible for people living in rural areas. The latter group of people is constantly at risk of contracting malaria and it is therefore very important that they are aware of the symptoms of malaria, the fact that it is curable, and that safe and effective treatment should be widely available.

Table 1.2 WHO recommended insecticides for indoor residual spraying against malaria vectors (WHO, 2009b)

Insecticide compounds and formulations (1)	**Class group (2)**	**Dosage (g/m^2)**	**Mode of action**	**Duration of effective action (months)**
DDT (Dichloro-diphenyl-trichloroethane) WP	OC	1-2	Contact	>6
Malathion WP	OP	2	Contact	2-3
Fenitrothion WP	OP	2	Contact & airborne	3-6
Pirimiphos-methyl WP & EC	OP	1-2	Contact & airborne	2-3
Bendiocarb WP	C	0.1-0.4	Contact & airborne	2-6
Propoxur WP	C	1-2	Contact & airborne	3-6
Alpha-cypermethrin WP & SC	P	0.02-0.03	Contact	4-6
Bifenthrin WP	P	0.025-0.05	Contact	3-6
Cyfluthrin WP	P	0.02-0.05	Contact	3-6
Deltamethrin WP & WG	P	0.02-0.025	Contact	3-6
Etofenprox WP	P	0.1-0.3	Contact	3-6
Lambda-cyhalothrin WP & CS	P	0.02-0.03	Contact	3-6

(1) EC = Emulsifiable concentrate;
CS = Capsule suspension;
SC = Suspension concentrate;
WG = Water dispersible granules; and
WP = Wettable powder.

(2) OC = Organochlorines;
OP = Organophosphates;
C = Carbamates; and
P = Pyrethroids.

1.4 The treatment of malaria

Treatment failure could be attributed to drug resistance. However, this is not always the case. Factors, such as incorrect dosing, non-compliance with the duration of the treatment regimen, poor drug quality, drug interactions and misdiagnosis, are but a few of many factors contributing to treatment failure. These factors contribute to the escalating drug resistance, due to the fact that the parasites are in all of these cases being exposed to sub-therapeutic drug levels (Boland, 2001:12; Molyneux *et al.*, 1999:238).

A large number of malaria events are treated in rural areas and remote villages, where there are limited facilities, if any. To ensure that these patients receive the best possible chances to survive, it is essential that the treatment regime is straightforward and effective (WHO, 2006:1).

Partial treatments (not the full course of treatment as proven to be effective) should never be given, once the decision to give antimalarial treatment has been made. Even when a patient is considered to be semi-immune, or where the diagnosis is uncertain, a full course treatment should be administered and completed, once initiated (WHO, 2006:27).

HIV patients, who contract malaria, should receive standard antimalarial treatment regimens, as recommended in the relevant sections of the WHO guidelines for the treatment of malaria (WHO, 2006:39).

However, treatment, or intermittent prophylactic treatment with sulfadoxine/pyrimethamine, should not be given to HIV infected patients receiving co-trimoxazole (trimethoprim/sulfamethoxazole) prophylaxis (WHO, 2006:39).

1.4.1 Products for the treatment of malaria

The concurrent use of two antimalarials, having different mechanisms of action, have the potential to delay the development of resistance, due to the fact that the parasite might be resistant to one of the two components, but may be dispatched by the other (Boland, 2001:10).

In order to enhance the treatment outcome and prevent further resistance to monotherapies, the WHO is now recommending the use of antimalarial combination treatments for the treatment of *falciparum* malaria (WHO, 2006:17).

When the drug targets in the malaria parasite are linked to and/or depend on the synergy of the two components, as is the case in combinations, such as sulfadoxine/pyrimethamine, sulfalene/pyrimethamine, proguanil/dapsone, chlorproguanil/dapsone and atovaquone/proguanil, such combinations are considered as single products (WHO, 2006:16-17).

When a non-antimalarial medicine is included in multi-drug therapies, e.g. chlorpheniramine in the combination chloroquine and chlorpheniramine, it is not considered an antimalarial combination therapy (WHO, 2006:16-17).

1.4.1.1 Artimesinin-based combination therapy (ACT)

The artemisinins and partner medicines of ACTs should not be available as monotherapies, since combination therapy is more effective and reduces the chance for resistance against antimalarials.

Artemisinin and its derivatives (artesunate, **artemether**, artemotil, dihydroartemisinin) reduce the parasite numbers by a factor of approximately 10 000 (much higher than other current antimalarials) in each asexual cycle, hence, with a factor of about a hundred million during two asexual life cycles upon three days of treatment. This leads to rapid clearance of parasitaemia and quick eradication of associated symptoms. Artemisinin and its derivatives are rapidly eliminated, therefore a seven-day treatment course is required when given in combination with other rapidly eliminated compounds, such as tetracyclines and clindamycin. When given with slowly eliminated antimalarials (e.g. **lumefantrine**), a shorter course of only three days will be effective, since the partner medicine will “protect” artemisinin from resistance, by completely clearing the body of the remaining parasites. In return, artemisinin will also “protect” the partner medicine against resistance (WHO, 2006:16-17).

ACTs currently being recommended include:

- Artemether/lumefantrine.
- Artesunate + amodiaquine.
- Artesunate + mefloquine.
- Artesunate + sulfadoxine/pyrimethamine.

- Amodiaquine + sulfadoxine/pyrimethamine may be considered as an interim option, where ACTs cannot be made available (WHO, 2006:21).[1]

The mechanism of action for both artemether and lumefantrine will be discussed in Chapters 3 and 4.

1.4.1.2 Exclusion of certain antimalarial products

- Chloroproguanil/dapsone: Not yet evaluated as an ACT partner, thus insufficient evidence of efficacy and safety for recommendation as a combination partner exists (WHO, 2006:21).

- Atovaquone/proguanil: Although it has been proven to be safe and effective as combination partner, the high costs have prevented it from being recommended for use (WHO, 2006:22).

- Halofantrine: Not yet evaluated as an ACT partner and not recommended, because of safety concerns (WHO, 2006:22), such as ventricular arrhythmia causing death, potential embryo toxicity, etc. (Hardman *et al.,* 1996:975).

- Dihydroartemisinin (artenimol)/piperaquine: Has been proven to be safe and effective in trials in Asia, but has not yet been evaluated sufficiently in Africa and in South America (WHO, 2006:22).

In a randomised trial with combination therapy for uncomplicated *falciparum* malaria in Ugandan children, the combination of artemether/lumefantrine proved to be a highly efficacious treatment (Dorsey *et al.*, 2007:2210).

In another trial in India, artemether/lumefantrine proved to be safe and effective for the treatment of acute uncomplicated *falciparum* malaria, and also as a viable alternative for artesunate plus sulfadoxine/pyrimethamine (AS + SP). It should, however, be used with caution and its efficacy monitored periodically (Valecha *et al*., 2009:107).

[1] **/** indicates combination in one dosage form
+ indicates combination in two dosage forms

1.4.2 Resistance to antimalarial products

In the late 1950s, *Plasmodium falciparum* started showing resistance to chloroquine. In most geographical areas this resistance has continued to increase. In 1991, resistance to other antimalarials was detected in several continents, thus posing a serious risk (Wernsdorfer, 1991:297).

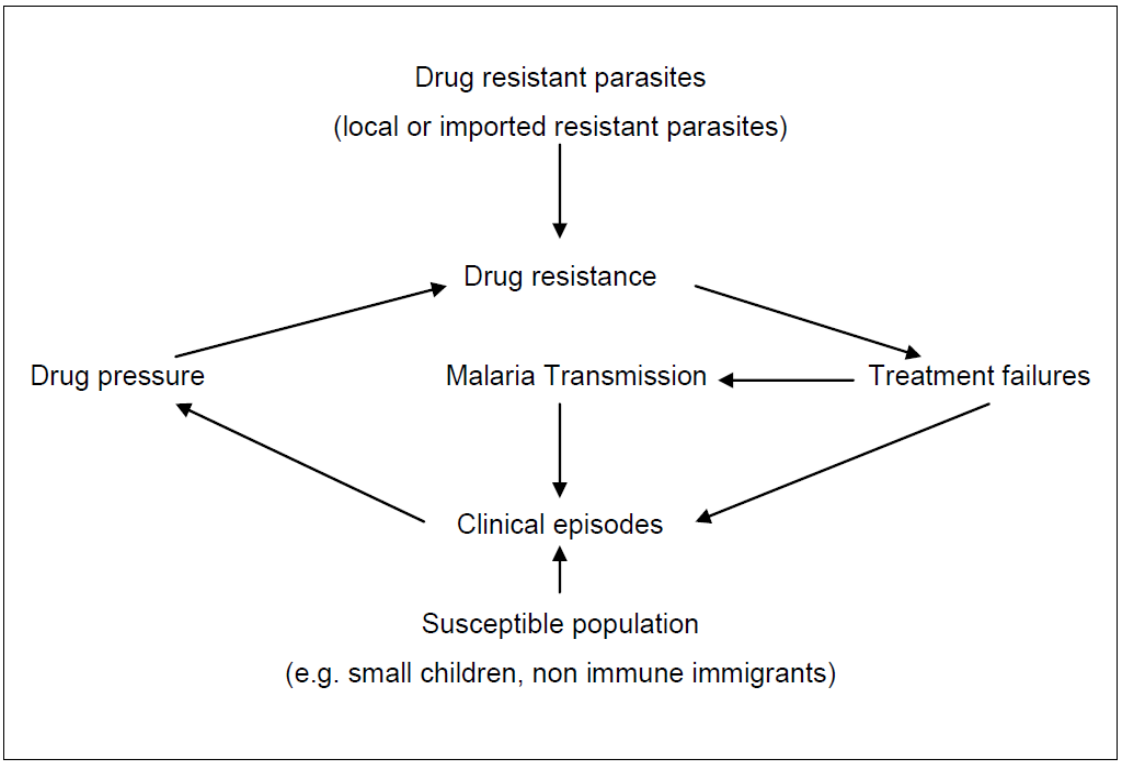


Figure 1.3 Emergence and spread of drug resistant *Plasmodium falciparum* (adopted from Bjorkman, 2002:1640).

Figure 1.3 shows the evolution of antimalarial resistance under drug pressure, as has been the case with chloroquine and which then started to happen rapidly with sulfadoxine/pyrimethamine, when used on a larger scale (Bjorkman, 2002:1640).

Resistance seemingly occurs through spontaneous mutations that lead to reduced sensitivity to certain drugs. If these mutations do not influence the survival/reproduction of the parasite, they will survive drug pressure (only the susceptible parasites will be removed). Previously, it has been found that in the same geographical area, drug susceptibility had shown a range from highly resistant to completely sensitive. However, a population will exhibit stable resistance over a period of time, even long after removal of drug pressure (Boland, 2001:12).

1.5 Availability and quality of antimalarial medicines

Cohen-Kohler (2007:610) state that "Pharmaceuticals are one of the cornerstones of human development, as their rational consumption can reduce morbidity and mortality rates and enhance the quality of life." Access to safe and effective medicines can thus make the difference between life and death. A third of the world's population, with high incidences in some rural parts of Asia and Africa, however, lacks regular access to essential medicines (Cohen-Kohler, 2007:610).

Buabeng *et al*. (2008:613) recently investigated the problems associated with the availability, distribution and choice of antimalarials at 130 licensed medicine outlets in Africa. The outcomes of this study revealed that recommended antimalarials had not been readily available at most of the accessible outlets.

In two rural districts of Tanzania, only 22.5% of children and 10.5% of adults had received prompt and appropriate antimalarial treatment during the course of a survey by Hetzel *et al.* (2008:317).

In addition, there is a high incidence of substandard medicines being available in a number of developing countries. In many instances, counterfeiting was suspected. In one study, 96 samples of chloroquine and selected antibacterials were collected from Nigeria and Thailand in a controlled and methodical manner. These samples were analysed by high performance liquid chromatography (HPLC), using appropriate validated methods. The results indicated that 36.5% of the products were substandard with respect to the pharmacopoeial limits. In a number of samples, decomposition had been the cause of the poor quality. However, it mostly appeared that poor manufacturing had been at the root of these inferior products (Shakoor *et al.*, 1997:839).

The WHO considers equitable access to safe and affordable medicines as vital to the attainment of the highest possible standard of health by all (WHO, 2009c). Quality assurance, with objective information of drugs, is vital to guarantee beneficial and cost-effective outcomes for the use of pharmaceuticals (USAID, 2007).

Recent reports revealed that the quantity of substandard and counterfeit medicines had reached alarming numbers in low income countries (USAID, 2007). This is disturbing, seeing that the availability of medicines in these countries is already a problem and since these countries have the largest number of infections.

1.6 Counterfeit and adulterated antimalarial medicines

Counterfeit medicines are defined as pharmaceutical products, containing no active ingredient, an incorrect amount of active ingredient, incorrect ingredient, and/or unapproved labeling and packaging. These products represent an unquantified problem of international concern (Ziance, 2008:71).

Products or medicines will be branded as adulterated when a mixture with, or substitution of another substance is:

1) Mixed or packed therewith, so as to reduce the quality or strength, or

2) Substituted wholly, or in part thereof (US FDA, 2009b).

In Cameroon, 284 antimalarials were sampled from 132 vendors. 32% of chloroquine, 10% of quinine and 13% of sulfadoxine/pyrimethamine proved to be probable counterfeit samples. Some of the fake quinine contained chloroquine. 6 out of 15 self medicated, malaria infected patients received chloroquine samples, containing no active ingredient, whereas 1 contained chloroquine lower than the expected content (Newton *et al.*, 2006a:604).

Suspiciously inexpensive mefloquine and artesunate samples were discovered in Cambodia, where chloroquine and sulfadoxine/pyrimethamine had been found ineffective. Of these, the “cheaper” artesunate products contained 6% of chloroquine and no artesunate, while the mefloquine products contained sulfadoxine/pyrimethamine and no mefloquine. A survey of 133 drug vendors and pharmacies suggested that, of the available artesunate and mefloquine, 71% and 60%, respectively, were fake drugs (Newton *et al*., 2006a:604-605).

In most instances, counterfeit medicines are presented in packaging that doesn’t look suspicious (Figures 1.4 and 1.5) and even have holograms that are very similar to the true product, as seen in Figure 1.6. In the example, each hologram is about 13 mm in diameter. Figure 1.6 shows the genuine Guilin Pharmaceutical Co Ltd hologram (left), showing X-52 code visible under UV light (top right), and a counterfeit type 9 hologram (right).

Figure 1.4 Genuine (left) and counterfeit (right) Cotecxin tablets (dihydroartemisinin) from Tanzania (Newton *et al.*, 2006b:752).

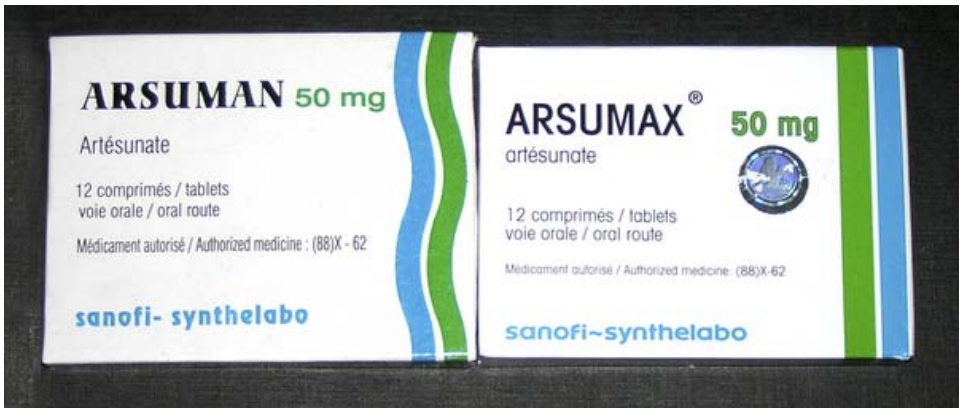


Figure 1.5 Genuine (right) and counterfeit (left) Arsumax tablets (artesunate) from Cameroon (Newton *et al.*, 2006b:753).

Figure 1.6 Examples of genuine (left) and counterfeit (right) artesunate holograms (Newton *et al*., 2006a:604).

In many countries, patients and antimalarial retailers are unaware of the existence of counterfeit, or substandard medicines. In Laos, 63% of antimalarial retailers and 80 - 96% of consumers were unaware of these products. Governments and companies have started to issue warnings against specific counterfeited products (Figure 1.7), whilst some countries, such as Nigeria and Thailand, have developed informative websites, providing valuable information to patients and antimalarial retailers (Newton *et al.,* 2006a:609).

Figure 1.7 A Khmer language poster warning of counterfeit artesunate and mefloquine tablets (Newton *et al.*, 2006a:609).

In May 2009, the National Agency for Food and Drug Administration and Control (NAFDAC) intercepted a consignment containing counterfeit and adulterated Maloxine® and Amalar® tablets. Laboratory testing of these tablets revealed that they contained sulfadoxine, but no pyrimethamine. These tablets, with an estimated street value of N32.1 million, would have led to hundreds of Nigerians suffering from treatment failure and complications, such as anaemia and drug resistance (Ogundipe, 2009).

In 2006, Newton *et al.* (2006a:602-613) indicated that internationally, eight of the twelve major antimalarials being used, had been counterfeited at some point. It seems that antimalarials are particularly targeted, due to its high volume demand. The increase in counterfeit antimalarials in third world countries has detrimental effects on public health, hence health authorities are urgently in need of effective methods for the detection of counterfeit medicines.

1.7 Conclusion

Numerous organisations are currently contributing to assist in the fight against malaria in tropical and sub-tropical areas, internationally.

Currently, the focus is on preventing the contracting of malaria, the early and accurate diagnosis thereof and ensurance of the availability of effective treatment for patients having malaria. This is very challenging, since most of the affected areas are rural and access to timely and efficient medical care is but one of many obstacles.

Furthermore, the problem of counterfeit medicines being distributed at an alarming rate, plays a major role in the fight against malaria.

Headlines, such as “Fake drugs kill 700 000”, “Interpol seizes $6.65 million in counterfeit HIV/AIDS, malaria and TB drugs in Southeast Asia”, “The morally uncomfortable global drug gap” and “Spread of malaria feared as drug loses potency” in major newspapers, journals and on websites, are becoming more regular, as the problem of counterfeit medicines continues to rise.

When infected patients receive these substandard or counterfeit antimalarials, it increases the development of resistance against the few effective drugs available, and creates a huge problem in the struggle against malaria.

Artimesinin-based combination therapy, including artemether/lumefantrine, as investigated during this study, forms part of the current, most effective and less expensive treatment regimes for the treatment of malaria.

In order to ensure that safe and effective antimalarials are available, measurements and regulations need to be available and supported by local governments. This is discussed further in Chapter 2.

CHAPTER 2

Considerations with regards to monograph development

2.1 Introduction

Professor Kristensen (2004:2), referring to the question as to why we still need a pharmacopoeia, said: "The answer can be understood only when we realise that the pharmacopoeia is no longer a formulary, but a collection of quality standards presenting the acceptable quality of pharmaceutical substances and finished drug products and the analytical means to assess their quality. The promotion of public health is still the objective of the pharmacopoeia, but today this role is shared with the licensing and inspecting authorities."

In Chapter 1, several challenges regarding malaria were introduced. These included the complexity of the disease's pathology, its geographic distribution, the unavailability of safe and effective medication in the areas affected, the development of resistance, as well as substandard/counterfeit medicines. The importance of good quality control of the few effective antimalarial medicines currently available was emphasised, as part of ensuring their long-term efficacy. Hence, a need was identified by the WHO's Expert Committee regarding the development of specifications for pharmaceutical preparations, for inclusion in the monographs of lumefantrine and artemether/lumefantrine combination products, which currently play an integral role in the fight against malaria.

Monographs in the International Pharmacopoeia (Ph.Int.) provide the quality dimensions for medicines, included on the basis of efficacy and safety, on the Essential Medicines List (EML) and in WHO treatment guidelines (WHO, 2010). The Research Institute for Industrial Pharmacy (RIIP®), incorporating the Centre for Quality Assurance of Medicines (CENQAM®), a WHO Collaborating Centre, was requested by the WHO to assist in developing monographs for the antimalarial, active pharmaceutical ingredient (API), lumefantrine, as well as for the fixed-dose-combination (FDC) products of artemether/lumefantrine, including FDC tablets and powders for suspension.

The model for monograph development, as depicted in Figure 2.1, was applied during this study. The 18 steps for monograph development, as stipulated by the Ph.Int. (WHO, 2010), were incorporated into this model.

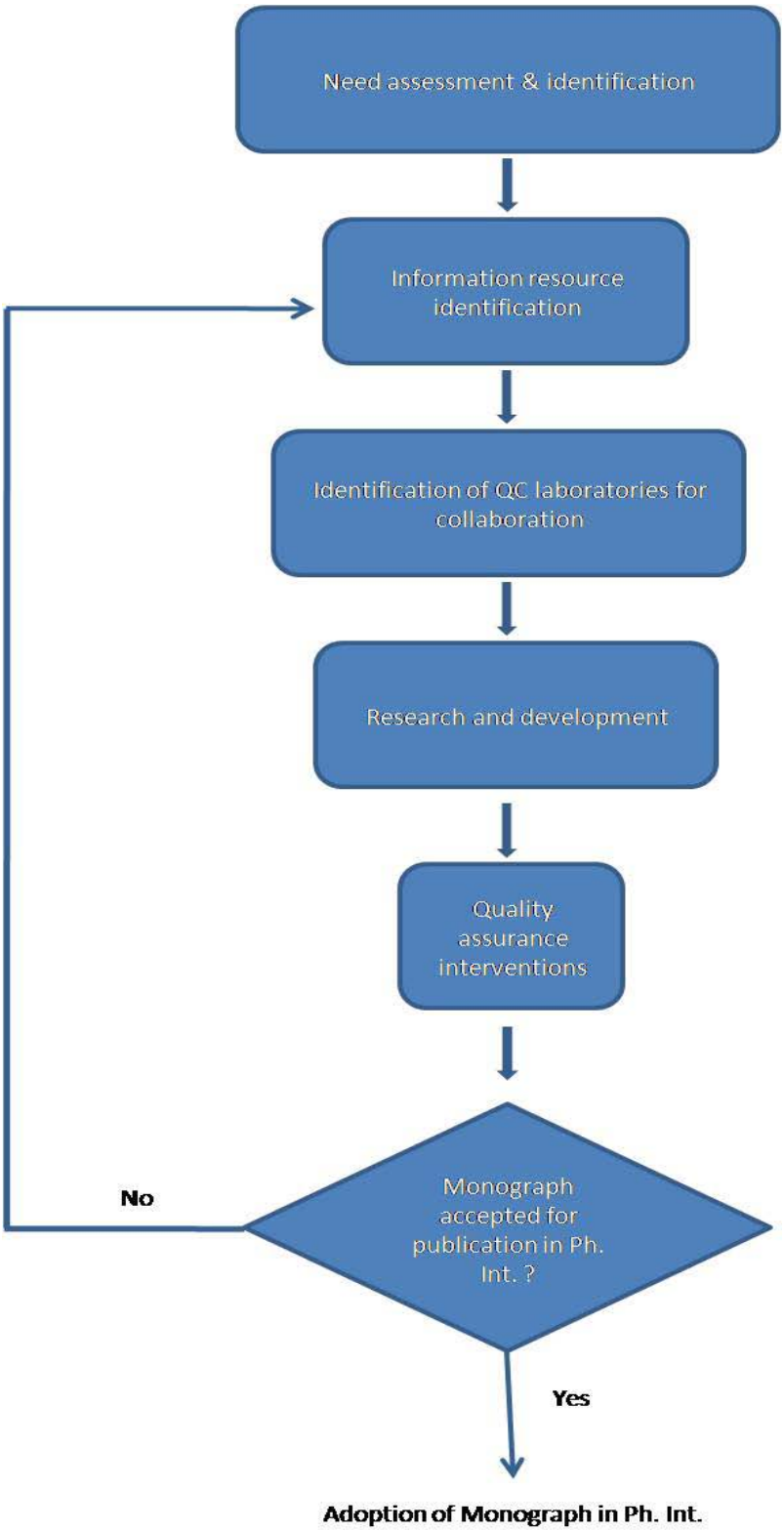


Figure 2.1 Model for monograph development.

2.2 Monograph development

The process for the development of monographs for the Ph.Int. is designed to ensure wide consultation and transparency and to make the adopted text available in a timely manner. It entails 18 steps, as discussed below (WHO, 2010).

Note: A "schedule for the adoption process", outlining the development history of a draft monograph, is included in each working document, which is circulated for comment.

- **Step 1:** Identification of specific pharmaceutical products for which quality control (QC) specifications need to be developed, confirmation by all WHO parties concerned (including the Department of Essential Medicines and Pharmaceutical Policies (EMP), specific disease programmes and the Pre-qualification Programme).
- **Step 2:** Provision of contact details from manufacturers of the above products, in collaboration with all parties concerned.
- **Step 3:** Contact manufacturers for provision of QC specifications and samples.
- **Step 4:** Identify and contact QC laboratories for collaboration in the project (2-3 laboratories, depending on how many pharmaceutical products have been identified in step 1).
- **Step 5:** Prepare the contract for drafting of the specifications and undertaking the necessary laboratory work.
- **Step 6:** Search for information on QC specifications available in the public domain.
- **Step 7:** Laboratory testing, development and validation of QC specifications.
- **Step 8:** Support WHO Collaborating Centre in the establishment of International Chemical Reference Substances.
- **Step 9:** Follow the consultative process, mailing of draft specifications to Expert Panel and specialists.
- **Step 10:** Discussion of comments with contact laboratories, WHO Collaborating Centers and additional laboratory testing to verify and/or validate specifications.

- **Step 11:** Consultation to discuss the comments and test results received as feedback.
- **Step 12:** Recirculation for comments.
- **Step 13:** As step 10.
- **Step 14:** Present the drafts of the WHO Expert Committee Specifications for Pharmaceutical Preparations for possible formal adoption. If not adopted repeat steps 11 to 13 as often as necessary. If adopted proceed to step 15.
- **Step 15:** Incorporate all changes agreed during discussion leading to adoption together with any editorial points.
- **Step 16:** Where necessary, also take account of any further comments that may still be received due to comment deadlines for recirculated texts (Step 12 and beyond) falling shortly after the meeting.
- **Step 17:** In all cases, confirm the amended test by correspondence with the relevant experts and/or contract laboratory before making it available on the WHO Medicines website.
- **Step 18:** Make "final texts" available on the WHO Medicines website to provide users, such as prequalification (PQ) assessors and manufacturers, with the approved specifications, in advance of the next publication date (WHO, 2010).

2.3 Structure of monographs in the International Pharmacopoeia

Monographs, currently developed and accepted for inclusion in the Int.Ph., contain specific information in a uniform structure. Table 2.1 summarises the different components, which API and dosage form monographs should have.

Table 2.1 A Summary of the basic components of monographs in the Ph.Int. (2008:5-360)

1. Components of API monographs
General information
➢ Name, structure, molecular formula, relative molecular mass, chemical name, synonyms; ➢ Description; ➢ Solubility; ➢ Category; ➢ Storage; and ➢ Additional information.
Requirements
➢ General; ➢ Definition; ➢ Identity test(s); ➢ Assay; ➢ Related substances/impurities; and ➢ Other (e.g. heavy metals, sulfated ash and loss on drying, or water).
2. Components of solid dosage form monographs
General information
➢ Category; ➢ Storage; and ➢ Additional information.
Requirements
➢ Definition; ➢ Identity test(s); ➢ Related substances/impurities; ➢ Assay; and ➢ Dissolution (more recently).

2.4 Guidelines for registration of fixed-dose-combination (FDC) products

The need for the development of fixed-dose-combination (FDC) dosage forms is becoming extremely important. FDC products are used for a wide range of treatments and are especially important for the treatment of infectious diseases, such as HIV/AIDS, malaria and tuberculosis, all of which are responsible for a large amount of illnesses and deaths around the world (WHO, 2005:95).

FDC products are of enormous advantage, if an identifiable patient population will benefit from a particular combination of actives in a fixed ratio, and if this specific combination is shown to be effective and safe. All of the actives should contribute to the overall therapeutic outcome (WHO, 2005:95).

FDCs also play an important role when limited resources are available (as is the case in most of the rural areas where malaria is a large threat) and when the cost of the FDC finished pharmaceutical product (FDC-FPP) is less than that of the individual products, when given concurrently (WHO, 2005:96).

Patient adherence is likely to be better and the chances of resistance against antimicrobials may also decrease with the use of FDCs (WHO, 2005:96).

Even though FDCs may have a lot of potential benefits, it cannot be assumed that the benefits will outweigh the risks. It is therefore of importance, as for any newly developed medicine, that the benefits and risks be compared and the FDCs proven to be safe and effective for the indications claimed for (WHO, 2005:96).

According to the WHO guidelines (WHO 2005:116-118), the quality data requirements for marketing authorisation/registration of FDC products include the following:

6.3.1 *In relation to quality, very similar principles apply to FDC-FPPs, as apply to single entity products. However, there are additional complexities arising from the need to consider two or more actives, instead of one. These complexities are principally, but not exclusively, related to assay, stability, physicochemical properties (for example dissolution rate) and bioavailability/bioequivalence.*

6.3.2 *Appendix 3, entitled Development (or Preformulation) Studies, makes some general points about this type of study. Pharmaceutical development studies are especially important for FDC-FPPs, because they are technically more*

demanding than single-component products. Issues that are specific to the development of FDC-FPPs include:

6.3.2.1 Chemical and physicochemical compatibility of the APIs in an FDC with one another, as well as with possible excipients.

6.3.2.2 The degradability of each API under stress conditions in the presence of the others.

6.3.2.3 Uniformity of content of each active, prior to compression (tablets), or filling (for instance capsules, sachets and suspension dosage forms). This study determines whether mixing during manufacturing is adequate.

6.3.2.4 Analytical procedures. These should be validated for each active in the presence of the others, during development of analytical methods for quality control of the finished product, stability testing and dissolution testing. Validation should be conducted for each active in the presence of the others and in the presence of related synthesis (process) impurities and potential degradation products. In case of HPLC (a common analytical technique), possible interference by degradation products in the assay of the active can usually be controlled by peak purity testing.

6.3.2.5 The dissolution rate of each active in pilot formulations. Multipoint limits should normally be established for routine quality control of each active. For some FDC-FPPs, different dissolution media may be acceptable for the different actives.

6.3.2.6 Different assay procedures may be necessary for the different actives in the finished product, and for different purposes (e.g. dissolution testing may be needed, rather than stability testing).

6.3.3 For solid dosage forms, a test and limit for content uniformity should be applied to any active that is present at a weight of ≤ 25 mg, or when the API comprises 25% or less of a dosage unit. Some authorities permit an exception for soft gelatin capsules that contain a solution of the API. Typically, when any one API is present at less than 25 mg, or less than 25% of the weight of a dosage unit, all of the actives are subjected to content uniformity testing. If a solid dosage form is not subject to content uniformity testing, for example because all of the actives are present at a weight of greater than 25 mg and greater than 25% of the weight of a dosage unit, there should be a test and limit for mass variation.

6.3.4 Acceptance criteria for impurities in FDC-FPPs should not be expressed with reference to parent API, and not with reference to the total content of APIs. If an impurity results from a reaction between two APIs, its acceptance limits should be expressed in terms of the API that represents the worst case. If available, a reference standard should be used to quantify the degradation product in percentage mass/mass with respect to the parent API. Alternatively, and if justified, other quantitative techniques that are described in Impurities in new drug products (revised) (ICH Q3B(R), 2003), may be applied.

Note: There should be an approximate mass balance. Together with the remaining active, degradants, expressed with reference to the parent compound, should sum to approximately 100% of initial strength.

6.3.5 The specifications and defining characteristics of the product should be based on the most vulnerable active. For example, expiry dates should be based on the stability of the least stable active.

6.3.6 In setting specifications, relevant pharmacopoeial monographs, WHO guidelines and ICH guidelines should be taken into account. For example, in the absence of a relevant WHO guideline, the ICH guideline, 'Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances' (1999), is a suitable source of guidance.

6.3.7 Specifications, in addition to those in pharmacopoeias, may be necessary for APIs in some cases, for example for particle size, residual solvents and synthesis-related impurities that are not covered by relevant monographs.

2.5 Validation of methods for monographs

"The objective of validation of an analytical procedure or method is to demonstrate that it [the analytical procedure or method] is suitable for its intended purpose." (ICH Q2(R1), 2005:1).

In order to ensure that a method is validated, each specific procedure/technique has certain parameters that need to be investigated upon validation. Table 2.2 summarises the parameters which should be investigated for each procedure, whilst Figure 2.2 illustrates the steps to be taken during the validation process. This is followed by a brief discussion of each of the validation parameters.

Table 2.2 Most important validation parameters for different types of analytical procedures (ICH Q2(R1), 2005:3)

Type of analytical procedure *Parameters*	**Identification**	**Testing for impurities**		**Assay** - ***Dissolution*** *(measurement only)* - ***Content/potency***
		Quantitative	*Limit*	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Intermediate precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection limit	-	- (3)	+	-
Quantitation limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this parameter is not normally evaluated.

+ signifies that this parameter is normally evaluated.

(1) In cases where reproducibility has been performed, intermediate precision is not required.

(2) Lack of specificity of one analytical procedure could be compensated for by another supporting analytical procedure(s).

(3) May be required in some cases.

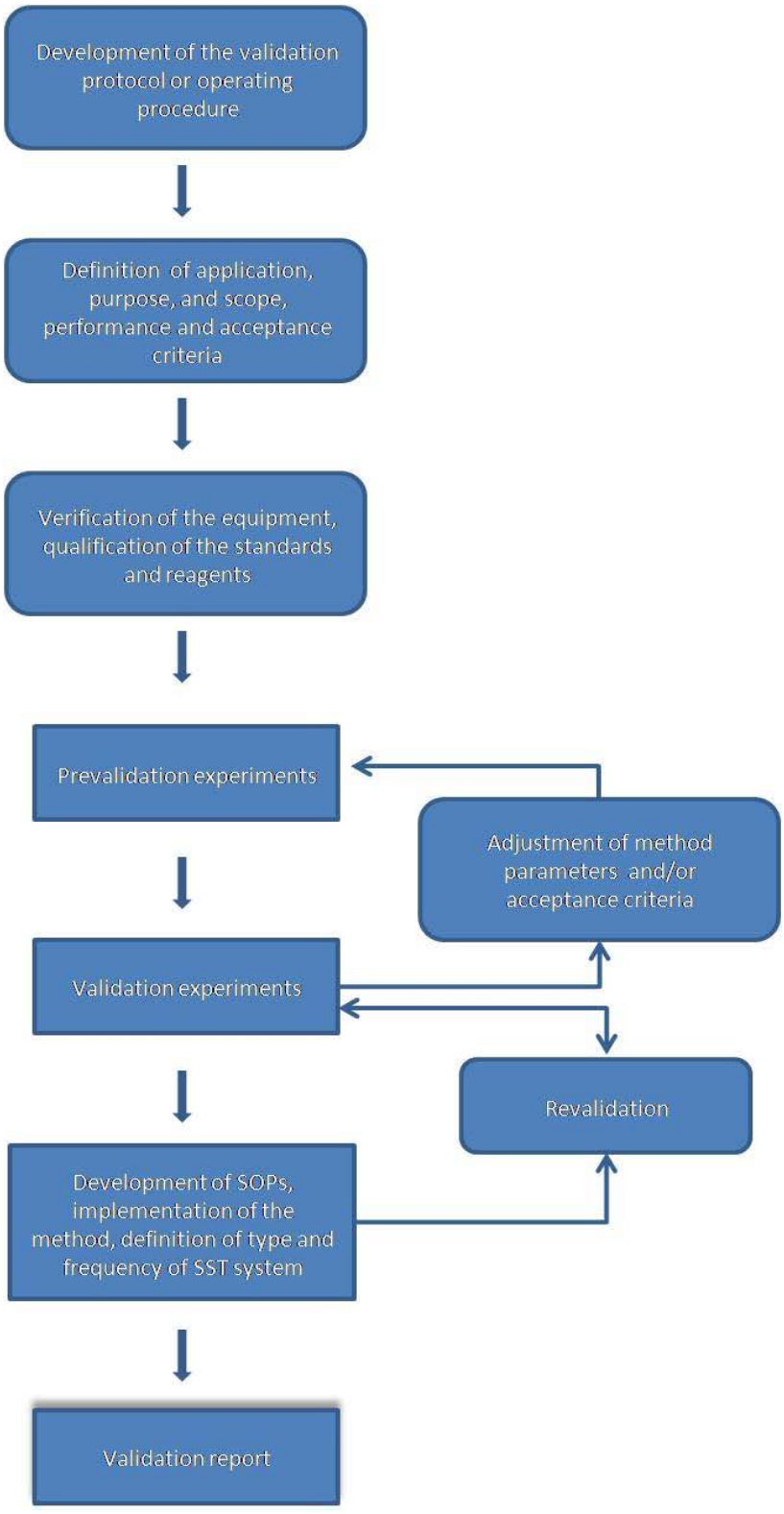


Figure 2.2 Steps taken during the validation of an analytical procedure/method (Yuwono & Indrayanto, 2005:246).

2.5.1 Specificity

The definition of specificity, according to ICH Q2(R1) (2005:4), is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present, such as impurities, degradants, matrix - and other active ingredients.

In analytical procedures, where it is impossible to demonstrate complete discrimination for a particular analyte, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination (ICH Q2(R1), 2005:4).

2.5.1.1 Identification

A suitable identification test(s) should be able to discriminate between different compounds, with closely related structures and a high probability of being present. A confirmation of the discrimination in samples, containing the analyte, may be verified by positive results, e.g. comparison with a known reference material. This may be coupled with negative results from samples that do not contain this specific analyte. In addition, the test may be performed on materials with a similar structure to that of the analyte, or closely related thereto, to confirm that a false positive result is not obtained.

The choice of these potential interfering substances should be considered, based on sound scientific judgment of the probability of this interference occurring (ICH Q2(R1), 2005:7). Infrared (IR) spectroscopy is seen as a specific identity test and may be used exclusively. Other techniques that make use of chromatographic procedures are not seen as specific, but may be used in combination, when the separation is based on different principles, such as chromatographic retention time and thin layer chromatography (TLC) (ICH Q6A, 2000:9).

2.5.1.2 Assay and impurity tests

In chromatographic procedures, as well as in other separation techniques, the representative individual components should be accurately identified and labeled accordingly to demonstrate specificity. For critical separations, specificity could be demonstrated by the resolution between the two components eluting close to each other. All critical separations in chromatography should be investigated at an appropriate level. When a non-specific method, for example a titration, is used to assay the API for release, other supporting analytical procedures, like a suitable test for impurities, should be used in combination with the assay, in order to demonstrate overall specificity. The specificity of assay and impurity tests are approached similarly (ICH Q2(R1), 2005:7).

- **If impurities[1] are available**

In case of assay, discrimination of the analyte in the presence of impurities, other APIs (in the case of FDCs) and/or excipients, should be demonstrated. For example, spiking of pure substances (API or dosage form) with appropriate levels of impurities and/or excipients and comparing the results to unspiked samples, in order to demonstrate that the assay result is affected by the presence of these materials.

For testing of the impurities, the product/substance could be spiked with appropriate levels of impurities to demonstrate separation of the impurities from each other, as well as from other components present (ICH Q2(R1), 2005:7).

- **If impurities are unavailable**

Where impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples, containing impurities or degradation products, to a second, well characterised procedure, e.g. a pharmacopoeial method, or other validated analytical procedure (independent procedure). These samples should include storage under relevant stress conditions, such as light, heat, humidity, acid/base hydrolysis and oxidation.

- For the assay, the two results should be compared.

- For the impurity tests, the impurity profiles should be compared.

Peak purity tests (e.g. diode array, mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component (ICH Q2(R1), 2005:7).

2.5.2 Linearity

The ICH Q2(R1) (2005:5) defines the linearity of an analytical procedure as its ability, within a given range, to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

A linear relationship should be evaluated across the range (see 2.5.3) of the analytical procedure. It may be demonstrated directly on the API (by dilution of a standard stock solution) and/or by weighing separate mixtures of the pharmaceutical product components, following the proposed procedure (this can be included during investigation of the range) (ICH Q2(R1), 2005:8).

[1] Impurities ≡ related substances

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration, or content. If there is a linear relationship, appropriate statistical methods, e.g. calculation of a regression line by means of least squares, should be used to evaluate results. In order to obtain linearity between assays and sample concentrations, results may in some cases need to be subjected to mathematical transformation, prior to the regression analysis. The degree of linearity may be presented by the mathematical estimates of data from the regression line (ICH Q2(R1), 2005:8).

The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be calculated. A plot of the data should be included in the validation report. Additionally, an analysis of the deviation of the actual data points from the regression line, may also be helpful in the evaluation of linearity. A minimum of 5 concentrations are recommended for the establishment of linearity. Any other approach should be justified (ICH Q2(R1), 2005:8; Yuwono & Indrayanto, 2005:249-250).

2.5.3 Range

The range of an analytical procedure is defined as the interval between the upper and lower concentration (amounts) of analyte in the sample, including those concentrations, for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity (ICH Q2(R1), 2005:5).

The specific range for an analytical procedure is normally a result of the linearity studies and the intended application of the procedure. The results, produced by an analytical procedure, should supply an acceptable degree of linearity, accuracy and precision for samples containing amounts of analyte within the limits, or at the extremes of the specified range of the analytical procedure. Table 2.3 illustrates the minimum, specified ranges to be considered for analytical tests (ICH Q2(R1), 2005:8).

Table 2.3 The minimum specified ranges to be considered for each test (ICH Q2(R1), 2005:9; USP, 2005:2751)

Test	Recommended range
Assay	80% -120% of test concentration
Uniformity of content	70% - 130% of test concentration (unless a wider/more appropriate range is justified)
Dissolution	± 20% over the specified range
Impurities/related substances	From the reporting level of the impurity to 120% of the specification

For impurities, which may be unusually potent, or produce toxic, or unexpected pharmacological effects, the detection/quantitation limit should be proportionate to the control level of the impurities (ICH Q2(R1), 2005:9).

Where the assay and purity are performed collectively as one test and only a 100% standard is used, linearity of the standard should cover the range from the reporting level of the impurities to 120% of the assay specification (ICH Q2(R1), 2005:9).

2.5.4 Accuracy

The ICH Q2(R1) (2005:9) defines accuracy of an analytical procedure as the expression of the closeness of agreement between the value, which is accepted either as a conventional true value, or an accepted reference value and the value found.

Accuracy should be established across the specified range of the analytical procedure.

2.5.4.1 Assay

Active pharmaceutical ingredient (API)

Several methods to evaluate accuracy are available:

- Application of an analytical procedure to an analyte of known purity (e.g. reference material);
- Comparison of the results of the proposed analytical procedure with those of a second, well characterised procedure, the accuracy of which is stated and/or defined (independent procedure, see 2.5.1.2.); and

- Once precision, linearity and specificity have been established, accuracy may be inferred (ICH Q2(R1), 2005:9).

Pharmaceutical product

- Methods to evaluate that the accuracy of an analytical method is the same as for the APIs, except that instead of the analyte, synthetic mixtures (analytical placebo) of the product's components, to which known quantities of the API to be analysed have been added, are used.
- In cases where it is impossible to obtain samples of all the FPP components, it may be acceptable to either add known quantities of the analyte to the finished product, or to compare the results obtained from a second, well characterised procedure, the accuracy of which is stated and/or defined (independent procedure, see 2.5.1.2.) (ICH Q2(R1), 2005:9).

2.5.4.2 Impurities (quantitation)

Accuracy should be assessed on samples (API/pharmaceutical product), spiked with known amounts of impurities.

In cases where it is impossible to obtain samples of certain related substances, it is considered acceptable to compare results obtained by an independent procedure (see 2.5.1.2.). In these cases the response factor of the active substance may be used.

It should be clear how the individual, or total impurities are to be determined, e.g. mass/mass, or area percent, in all cases with respect to the major analyte (ICH Q2(R1), 2005:10).

2.5.4.3 Recommended data for validation report

Accuracy should be assessed, using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (e.g. three concentrations/three replicates each of the total analytical procedure).

Accuracy should be reported as percentage recovery by the assay of known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with the confidence intervals (ICH Q2(R1), 2005:10).

2.5.5 Precision

As defined by the ICH, the precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels, i.e. repeatability, intermediate precision and reproducibility (ICH Q2(R1), 2005:4).

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

2.5.5.1 Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time and is also termed, intra-assay precision (ICH Q2(R1), 2005:5).

Repeatability should be assessed using:

a) A minimum of 9 determinations covering the specified range for the procedure (e.g. three concentrations and three replicates each); or

b) A minimum of 6 determinations at 100% of the test concentration (ICH Q2(R1), 2005:10).

2.5.5.2 Intermediate precision

Intermediate precision expresses within-laboratories variations, such as different days, different analysts and different equipment (ICH Q2(R1), 2005:5).

The circumstances and intended purpose of an analytical procedure will determine the extent to which intermediate precision should be established. The effects of random events on the precision of the analytical procedure should be established. Typical variations to be studied, separately or together, include days, analysts, equipment, etc. (ICH Q2(R1), 2005:10).

2.5.5.3 Reproducibility

Reproducibility expresses the precision between laboratories, i.e. collaborative studies, usually applied to standardisation of methodology (ICH Q2(R1), 2005:5).

Reproducibility is assessed by means of an inter-laboratory trial and is considered to be very important in the process of standardising analytical procedures for inclusion in pharmacopoeias. This data is not part of the marketing authorisation dossier (ICH Q2(R1), 2005:10).

2.5.5.4 Recommended data for validation report

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision being investigated (ICH Q2(R1), 2005:10).

2.5.6 Detection limit

The ICH defines the detection limit of an analytical procedure as the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value (ICH Q2(R2), 2005:5).

The specific procedure, whether non-instrumental or instrumental, will lead the approach for determining the detection limit. Approaches other than those listed in Table 2.4 may be considered adequate (ICH Q2(R1), 2005:11).

Table 2.4 Different approaches for determining the detection limit (ICH Q2(R1), 2005:11)

Visual Evaluation
➢ Suitable only for instrumental and non-instrumental methods. ➢ Detection limit is determined by the analysis of samples with a known concentration of the analyte. ➢ Establishing the minimum level at which the analyte can be reliably detected.
Signal-to-Noise
➢ Suitable for analytical procedures that exhibit baseline noise. ➢ Determination of signal-to-noise ratio is done by comparing measured signals from samples with known low concentrations of the analyte to those of blank samples. ➢ Establishing the minimum concentration at which the analyte can be reliably detected. ➢ A signal-to-noise ratio between 3:1 or 2:1 is generally considered acceptable for estimating the detection limit.
Standard deviation of the response and the slope
➢ The detection limit (DL) may be expressed as: $DL = \frac{3.3\,\sigma}{S}$ Where: σ = the standard deviation of the response S = the slope of the calibration curve

2.5.6.1 Recommended data for validation report

The detection limit and the method used for determining it should be presented. If the detection limit is determined based on visual evaluation, or based on signal-to-noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification. Where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near, or prepared at the detection limit (ICH Q2(R1), 2005:11-12).

2.5.7 Quantitation limit

The quantitation limit of an individual analytical procedure is defined by the ICH as the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. It is a parameter of quantitative assays for low levels of compounds in sample matrices, used particularly for the determination of impurities and/or degradation products (ICH Q2(R1), 2005:5).

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is instrumental or non-instrumental. Approaches, other than those listed in Table 2.5 below, may be considered acceptable (ICH Q2(R1), 2005:12).

Table 2.5 Different approaches to determine the quantitation limit (ICH Q2(R1), 2005:12)

Visual Evaluation
➢ Suitable for instrumental and non-instrumental methods. ➢ The quantitation limit is generally determined by the analysis of samples with a known concentration of the analyte. ➢ Establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
Signal-to-Noise
➢ Suitable only for analytical procedures that exhibit baseline noise. ➢ Determination of signal-to-noise ratio is done by comparing measured signals from samples with known low concentrations of the analyte to those of blank samples. ➢ Establishing the minimum concentration at which the analyte can be reliably quantified. ➢ A typical signal-to-noise ratio is 10:1.
Standard deviation of the response and the slope
➢ The quantitation limit (QL) may be expressed as: $QL = \frac{10\,\sigma}{S}$ where: σ = the standard deviation of the response S = the slope of the calibration curve

2.5.7.1 Recommended data for validation report

The quantitation limit and the method used for determining it should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near, or prepared at the quantitation limit (ICH Q2(R1), 2005:13).

2.5.8 Robustness

The ICH guideline on the validation of analytical procedures defines the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage (ICH Q2(R1), 2005:5).

The evaluation of robustness should be considered during the development phase and will depend on the type and sensitivity of the procedure to be validated. It should demonstrate the reliability of a procedure with respect to deliberate variations in method parameters. If measurements are susceptible to certain variations in analytical conditions, these conditions should be suitably controlled, or precautionary statements be included in the procedure. One outcome of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used (ICH Q2(R1), 2005:13).

Table 2.6 Examples of typical variations for robustness testing in HPLC analysis (ICH Q2(R1), 2005:13)

High pressure liquid chromatography (HPLC)
➢ Influence of variations of pH in a mobile phase.
➢ Influence of variations in mobile phase composition.
➢ Different columns (different batches and/or suppliers).
➢ Temperature.
➢ Flow rate.
➢ Stability of analytical solutions.

2.5.9 System suitability testing

System suitability testing is an essential part of most analytical procedures. These tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed, constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. The pharmacopoeias contain recommendations regarding system suitability requirements (ICH Q2(R1), 2005:13). In

the Ph.Int., column performance (including efficiency, resolution factor, capacity factor and symmetry factor), relative retention and repeatability are among the factors being recommended for system suitability. The Ph.Int. also suggests the use of an instrument, equipped with a special pumping system, sufficient time for equilibrium of the mobile phase when a linear gradient method is used on an HPLC system, and the injection of a solvent (blank run) to identify any interfering peaks as part of the system suitability test (Ph.Int., 2006:1188-1192).

2.6 Conclusion

Each monograph, taken as a whole, should provide a reliable basis for making an independent judgment as to the quality of the substance, in the best interest of protecting the public, as per the British Pharmacopoeia (BP, 2005).

With validated methods in monographs, such as those in the Ph.Int. (2008), which are freely available and aiming to include the WHO essential drug list, a good basis can be created for the fight against substandard and counterfeit medicines, in order to help ensure safe and effective medicines for patients in malaria affected areas. The importance of developing and validating methods for the lumefantrine API and artemether/lumefantrine fixed-dose-combination products has become a necessity that, although only a small contribution to a larger project, cannot be ignored.

The WHO website (http://www.who.int/medicines/publications/pharmacopoeia) contains a schedule of monographs that require development and that are currently in the process of being developed. It is clear that the development of monographs for the Ph.Int. requires a scientific process in collaboration with the members of the WHO Expert Advisory Panel peer review. In the process of developing monographs for the lumefantrine API and artemether/lumefantrine FDCs during this study, each of the parameters in Table 2.2 was investigated, according to the steps as outlined in Figure 2.2. Methods to be developed and validated were chosen according to Table 2.1, to ensure that the basic components, as required by the Ph.Int. monographs, were included. Development and validation were done by using the ICH guidelines (section 2.5) for each of the methods for assay, related substances, etc.

As part of the monograph development, the physico-chemical properties of lumefantrine and artemether were investigated for possible polymorphic or pseudopolymorphic behaviour. This part of the development and an overview of the pharmacological properties of the two APIs are provided next in Chapter 3.

CHAPTER 3

Physico-chemical properties of artemether and lumefantrine

3.1 Introduction

The pharmaceutical problems and challenges, associated with polymorphism of an active pharmaceutical ingredient (API) and differences in physico-chemical properties, are common. These differences could affect the stability and quality of an API and can easily be detected with appropriate analytical methods (Abelli *et al.*, 2001:103).

Pharmaceutical solids exhibit polymorphism, when the same pure substance exists in two or more different crystalline phases, having different arrangements and/or conformations of molecules in the crystal lattice (Grant, 1999:2). Pseudopolymorphs, also known as solvates, are crystalline adducts, containing solvent molecules incorporated into the crystal structure, in either stoichiometric, or non-stoichiometric proportions, giving rise to unique differences in the physical and pharmaceutical properties of the APIs. If the incorporated solvent is water, the solvated form is called a hydrate (Vippagunta *et al.*, 2001:4).

Physical properties that may differ among various polymorphs include packing -, thermodynamic -, spectroscopic -, kinetic -, surface - and mechanical properties (Grant, 1999:7). These properties may directly impact on the APIs processability, the product manufacturability, the product quality and/or performance, which include stability, dissolution and bioavailability (Yu *et al.*, 2003:531).

It may therefore be necessary to include in a monograph, a specification regarding a certain polymorphic form to be used in a pharmaceutical product. Figure 3.1 (adapted from the ICH Q6A, 2000:Decision tree #4) provides a useful tool to determine the necessity of specifications when polymorphism exists, to be included in a monograph.

This chapter provides some pharmaceutical and pharmacological background on artemether and lumefantrine, whilst new, supplementary information, not previously published, is also presented with respect to possible polymorphic forms.

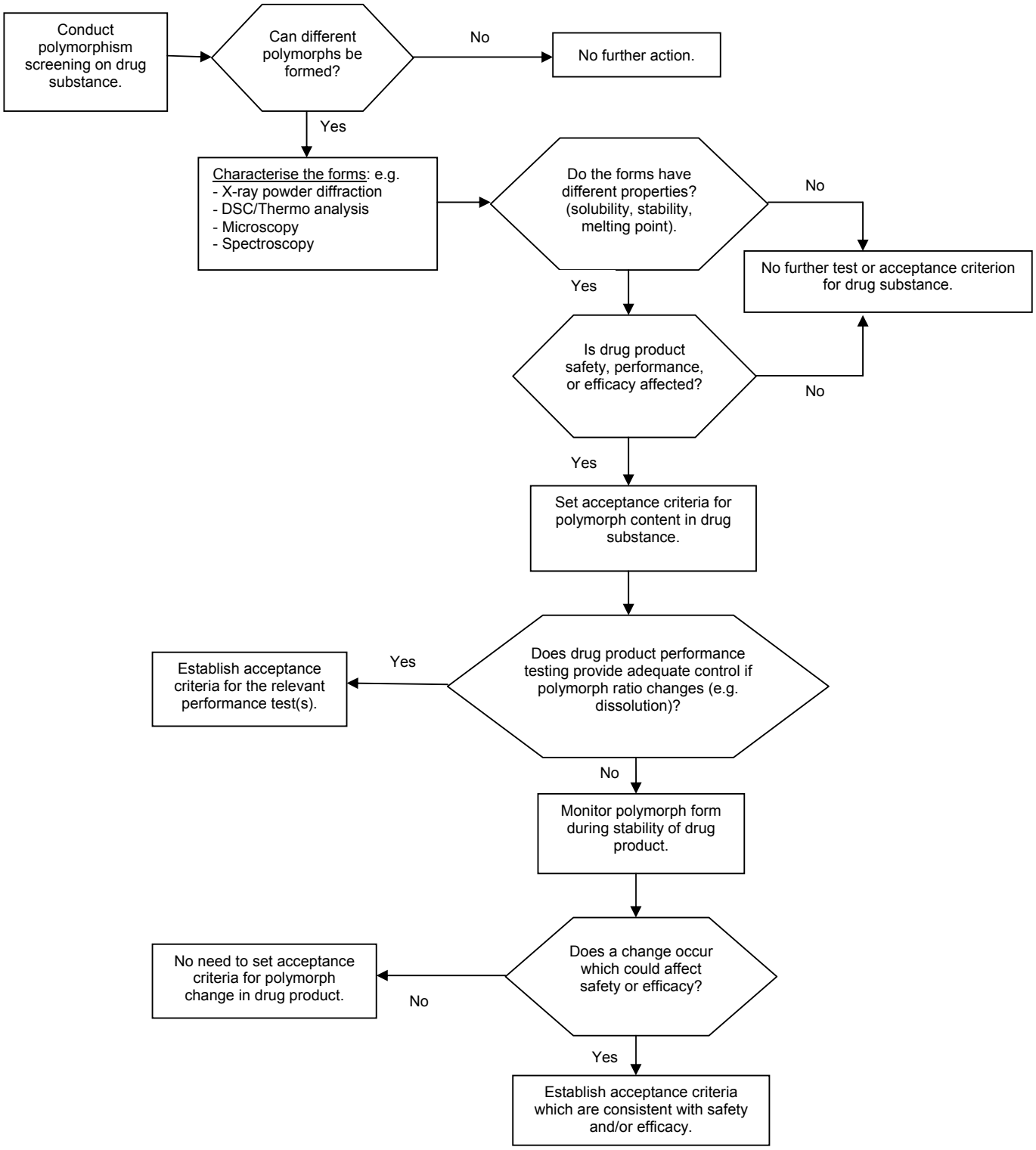


Figure 3.1 Condensed figure, diagrammatic representation illustrating the steps to be considered in polymorph studies during monograph development (adapted from the ICH Q6A, 2000:Decision tree #4).

3.2 Experimental conditions

3.2.1 Infrared (IR) spectroscopy

The IR spectra were recorded on a Nicolet Nexus 470-FT-IR spectrometer (Madison, Wisconsin, USA), over a range of 400 - 4000 cm^{-1}, using the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) method. Samples were prepared for DRIFT spectrometry by dispersing the sample in potassium bromide (KBr).

3.2.2 X-ray powder diffraction (XRPD)

The XRPD profiles were obtained at room temperature on a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2º/min (step size, 0.025º; step time, 1.0 sec). Approximately 200 mg of sample was transferred into an aluminium sample holder, taking care not to introduce a preferential orientation to the crystals.

3.2.3 Differential scanning calorimetry (DSC)

The DSC thermograms were recorded on a Mettler Toledo DSC823e instrument (Mettler, Switzerland). Samples weighing 3 - 5 mg were heated in sealed and pin-pricked aluminium crimp cells at a heating rate of 10ºC/minute, under a nitrogen gas flow of 10 ml/minute. The instrument was calibrated, using an ultra-pure indium standard, having a melting point of 156.4ºC. Samples were heated to a maximum temperature of 120ºC (artemether) and 200ºC (lumefantrine).

3.2.4 Particle size

Particle size distribution was measured with a Malvern Mastersizer 2000 (Malvern Instruments, UK), fitted with a Hydro 2000SM dispersion unit. Deionised water containing Tween 80 was used as dispersant.

3.2.5 Scanning electron microscopy (SEM)

Photomicrographs of different lumefantrine and artemether recrystallisation samples were recorded, using an FEI Quanta 200 ESEM & Oxford INCA 400 EDS microscope system (FEI Corporation, Hillsboro, USA). The lumefantrine samples were adhered to a small piece of carbon tape, mounted onto a metal stud (SEM pin) and coated with a gold-palladium film (Eiko engineering ion coater IB-2, Japan) in a vacuum. The artemether samples were adhered to a small piece of carbon tape, mounted onto a metal stud (SEM pin).

3.2.6 Thermal microscopy (TM)

Thermal microscopy was performed by preparing a small amount of substance on a microscope slide (MENZEL-GLASER microscope slide, 26 x 26 mm), covered with a covering glass (MENZEL-GLASER covering glass, 20x20 mm) and placed on the furnace. Heat was conducted through a Leitz heater and the temperature controlled by a Metratherm 1200d Ni Cr-Ni BBC GOERZ METRAWATT. The sample was enlarged by a Nikon ECLIPSE E400 light microscope and images stored, using a Nikon DS-Fi1 (Nikon, Japan) camera. The microscope was connected to the computer system through a Nikon Digital Sight USB(H) EXT. I/0. Images were analysed on the NIS-Elements F2.30 computer program.

3.2.7 Specific optical rotation

The specific optical rotation for lumefantrine was performed on a 0.05 g/ml solution of lumefantrine in chloroform. The angle of rotation of a neat liquid (α°) is the angle of rotation (α), expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at ambient temperature (20 – 25°C) using a layer of 1 dm. A Bellingham & Stanley ADP440 polarimeter (Bellingham & Stanley, UK) was used to perform the specific optical rotation.

3.3 Pharmaceutical and pharmacological background of artemether

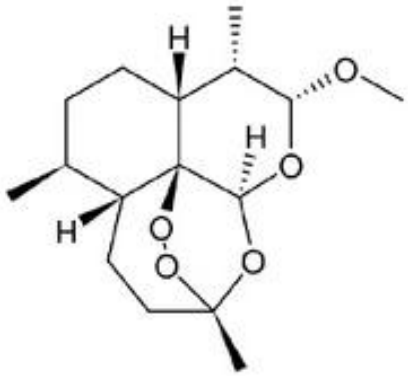
3.3.1 Description and nomenclature

White crystals, or white, crystalline powder.

Empirical formula: $C_{16}H_{26}O_5$

Molecular weight: 298.4

Structural formula:



Chemical name: (*3R,5aS,6R,8aS,9R,10S,12R,12aR)*-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-*12H*-pyranol[4,3-*j*]-1,2-benzodioxepin; CAS Reg. No. 71963-77-4

Nonproprietary name: Artemether.

3.3.2 Mechanism of action

Artemether is a synthetic analogue of artemisinin, which is a chemical extract from the Chinese herb, *Artemisia.* Artemisinin is a fast acting, blood schizonticide, which is effective in the treatment of acute malaria, including chloroquine-resistant and cerebral malaria. Artemether has higher activity and is better absorbed, compared to the poorly water soluble artemisinin. The compound is concentrated in parasite infested erythrocytes, and although the mechanism of action is unknown, it may involve rupturing of the parasite membrane.

This may be due to the carbon centered, free radicals, generated by the breakdown of ferrous protoporphyrin IX, or covalent alkylation of proteins. Artemether does not have any effect on the merozoites (hepatic phase) and is therefore of no use in chemoprophylaxis (Rang *et al.,* 2003:681).

3.3.3 Pharmacology

After rapid, but incomplete absorption of orally administered artemether, it is rapidly metabolised (*via* hydrolysis) to the demethylated derivative, dihydroartemisinin (DHA). Artemether and DHA reach peak concentrations within 2 - 3 hours after administration (Galichet *et al*., 2004:648, Djimdé & Lefèvre, 2009:2).

The elimination half-life is 1.5 - 3.5 hours for artemether and 1.4 - 2.4 hours for DHA. For patients with acute renal failure, the volume of distribution is about 3.2 - 6.9 l/kg and plasma clearance 5.4 - 13.8 ml/min/kg. For patients with normal renal function, the volume of distribution is 4.2 - 12.3 l/kg and the plasma clearance is 8.5 - 25.1 ml/min/kg. Artemether's binding to plasma proteins is about 50% (Galichet *et al*., 2004:648).

The administration of artemether to healthy volunteers, concurrently with a high-fat meal, increased the bioavailability two-fold, according to findings by Djimdé and Lefèvre (2009:2).

3.4 Physico-chemical properties of artemether

The artemether test sample that was used during this study was from Sinoway Industrial Co., Ltd., batch no. 060510. Artemether is practically insoluble in water, very soluble in dichloromethane and acetone, freely soluble in ethyl acetate and dehydrated ethanol (*Ph. Int.,* 2008).

3.4.1 Infrared spectroscopy (IR)

A sample of artemether was dispersed in KBr and the DRIFT spectrum recorded. The IR spectrum of artemether is shown in Figure 3.2 and the absorptions are listed in Table 3.1.

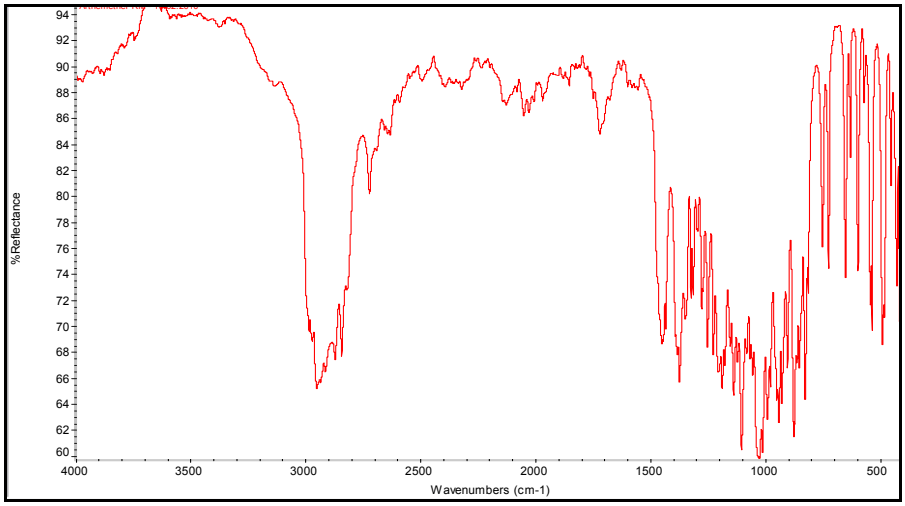


Figure 3.2 IR spectrum of artemether.

Table 3.1 Absorption peaks in the DRIFT-IR spectrum of artemether

Main absorptions	Wavenumbers (cm^{-1})	Main absorptions	Wavenumbers (cm^{-1})
1	631	22	1226
2	650	23	1251
3	726	24	1277
4	752	25	1295
5	814	26	1314
6	827	27	1323
7	852	28	1349
8	875	29	1374
9	905	30	1433
10	927	31	1451
11	941	32	1721
12	978	33	1854
13	991	34	1970
14	1013	35	2051
15	1026	36	2128
16	1056	37	2323
17	1104	38	2723
18	1122	39	2845
19	1137	40	2873
20	1153	41	2952
21	1189	-	-

3.4.2 X-ray powder diffraction (XRPD)

The main peak angles and relative intensities of artemether are listed in Table 3.2, and the XRPD pattern illustrated in Figure 3.3.

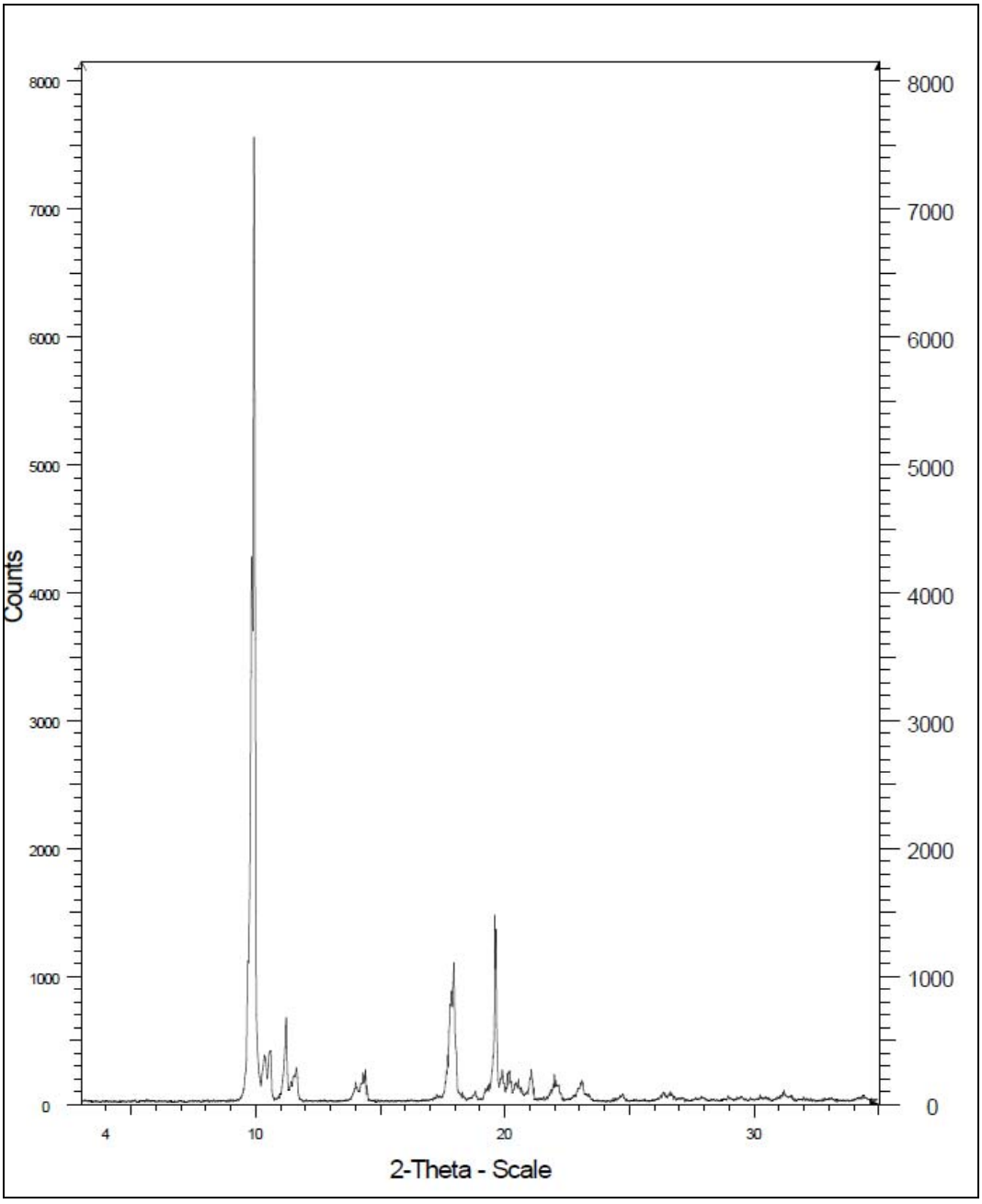


Figure 3.3 XRPD pattern of artemether.

Table 3.2 Main XRPD peaks and relative intensities of artemether

Peak angles (°2θ)	Relative intensities (I/I_0)
9.9	100
10.3	5
10.6	5
11.2	9
11.6	4
14.0	2
14.3	3
17.9	13
19.6	19
19.9	3
20.2	3
20.5	2
21.1	3
22.0	3
23.1	2

3.4.3 Differential scanning calorimetry (DSC)

The DSC trace of artemether (Figure 3.4) shows a melting endotherm at a temperature of 88.62°C.

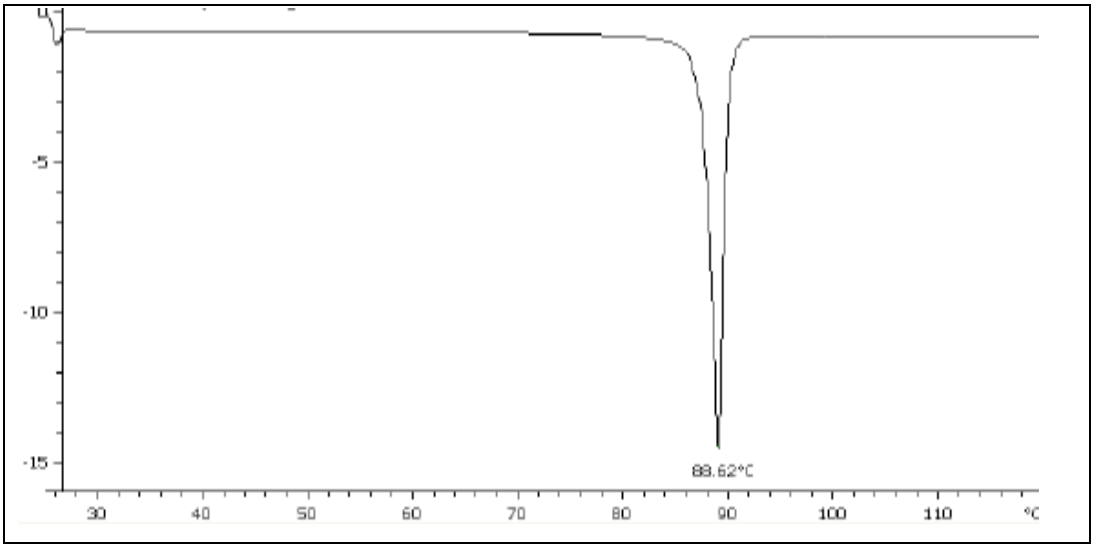


Figure 3.4 DSC trace of artemether.

3.4.4 Screening for polymorphism and pseudopolymorphism

Polymorphic and pseudopolymorphic screening during this study was performed according to the steps as outlined in Figure 3.1.

3.4.4.1 Recrystallisation method and organic solvents used

Saturated solutions of artemether were prepared in glass beakers or polytops by heating each solvent close to its boiling point, whilst continuously stirring with a magnetic stirrer and slowly adding artemether powder. The polytops or beakers were then sealed with perforated plastic caps or parafilm, allowing the solvent to slowly evaporate at room temperature.

The saturated solutions were prepared in the following organic solvents: acetone, acetonitrile, n-butanol, 2-butanol, dichloromethane (DCM), diethylether, dimethylformamide (DMF), ethanol, ethyl acetate, iso-propanol, methanol, n-propanol, tetrahydrofuran and toluene (Table 1, Appendix A).

3.4.4.2 Results and discussion

The IR spectra obtained for all the recrystallised samples were concordant to that of the artemether test sample (Figure 3.2). The IR spectrum of the recrystallisation product from ethanol (as an example) is shown in Figure 3.5.

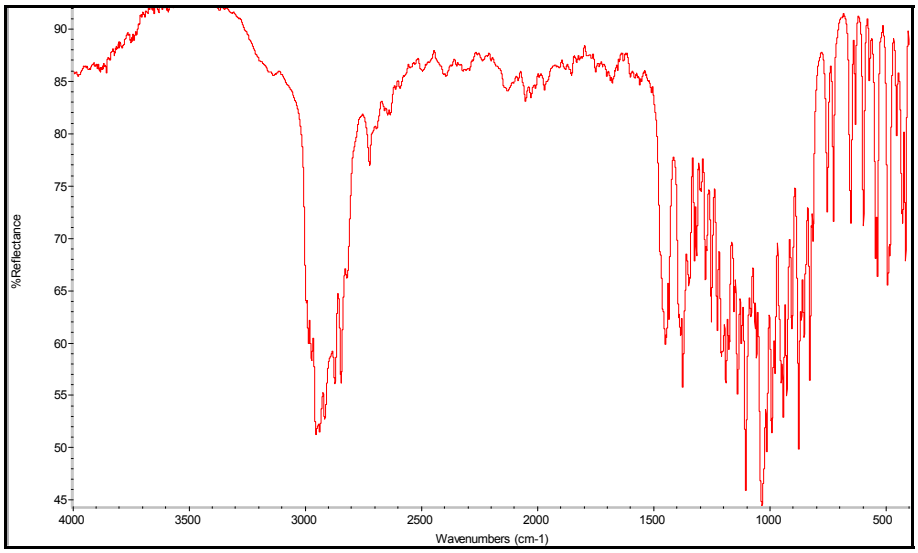


Figure 3.5 IR spectrum of artemether recrystallised from ethanol.

The recrystallisation products from the different organic solvents had endothermic melting peaks between 86°C - 89°C, corresponding to that of the artemether test sample (Table 3.3). The DSC trace shown in Figure 3.6 (recrystallisation from 2-butanol), represents the traces obtained for all the recrystallisation products.

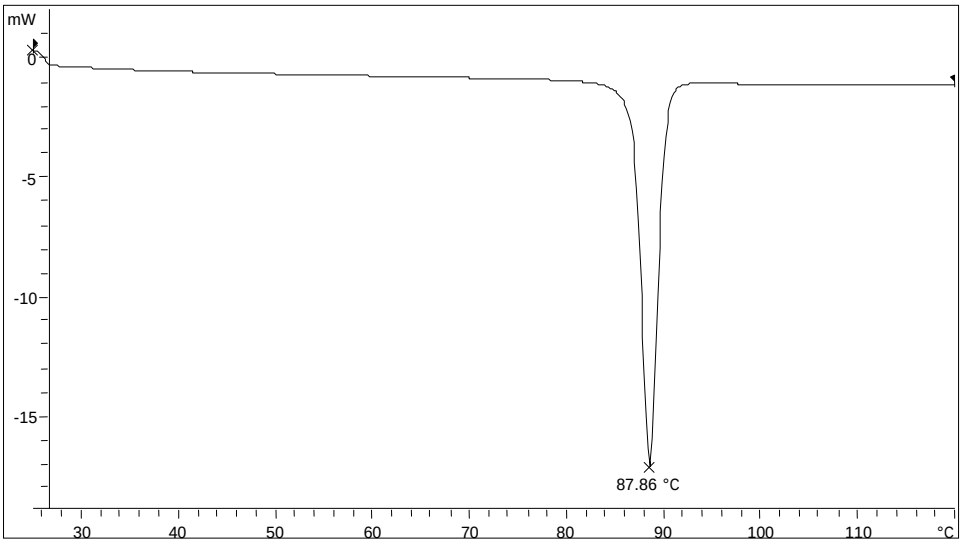


Figure 3.6 DSC trace of artemether recrystallised from 2-butanol.

Table 3.3 Summary of melting points for artemether crystals recrystallised from various solvents (determined by DSC)

Recrystallisation solvent	Melting point (°C)	Recrystallisation solvent	Melting point (°C)
Acetone	89.2	Ethanol	86.3
Acetonitrile	88.4	Ethyl acetate	89.7
n-Butanol	88.4	Methanol	87.8
2-Butanol	87.9	n-Propanol	87.7
Dichloromethane	87.9	Iso-Propanol	87.8
Diethylether	88.7	Tetrahydrofuran	88.6
Dimethylformamide	88.1	Toluene	88.0

The XRPD patterns of the crystals recrystallised from n-butanol and dimethylformamide (Figure 3.8), were not fully comparable to that of the artemether test sample, as seen in Figure 3.3. The overall patterns did not differ significantly, but small differences were observed in the 9 - 12 and 20 - 21 °2θ regions. The intensity of the peak at 11.2 °2θ for the test sample was higher (8.8 %) in comparison with those of n-butanol (0.8 %) and

DMF (1.8 %). Also, for the test sample, no peak was observed at 20.8 °2θ, compared to the n-butanol and DMF samples.

Although the XRPDs differed (Figure 3.8), the IR spectra and DSC thermograms of these two samples were concordant to that of the test sample. A difference in particles size and/or preferred orientation could have played a role in these minor differences observed in the XRPD patterns. SEM photomicrographs (Table 3.4) showed that all the recrystallisation products were poorly defined, plate-like aggregates. Davidovich e*t al.* (2005) demonstrated that the XRPD pattern of an anisotropic sample, along with different particle sizes, could be sufficiently influenced by preferred orientation, to have a distinct effect.

The XRPD patterns of all the other recrystallisation products were similar to that of the artemether raw material. The XRPD of the recrystallisation product from n-propanol is shown in Figure 3.7 as an example.

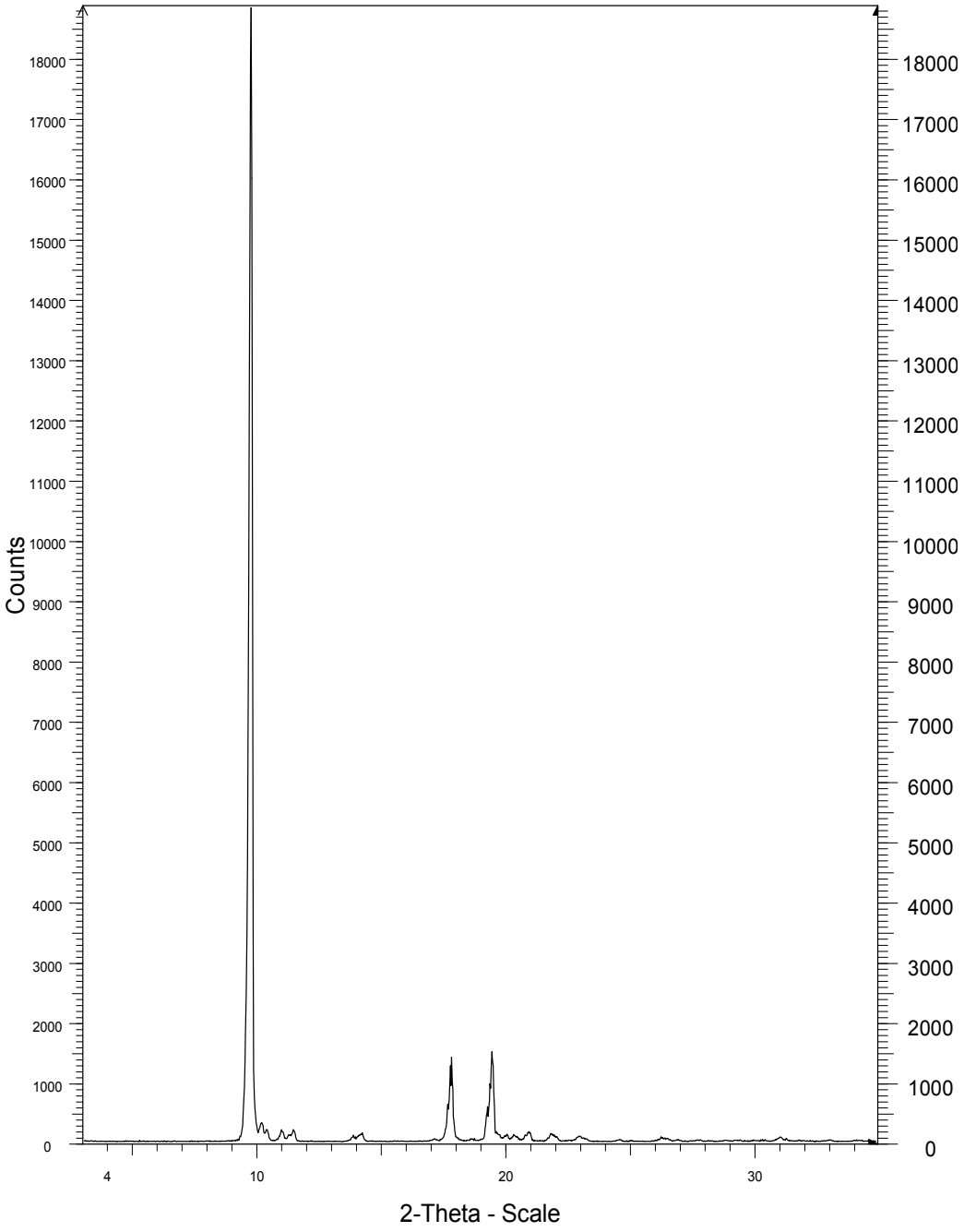


Figure 3.7 XRPD pattern of artemether recrystallised from n-propanol.

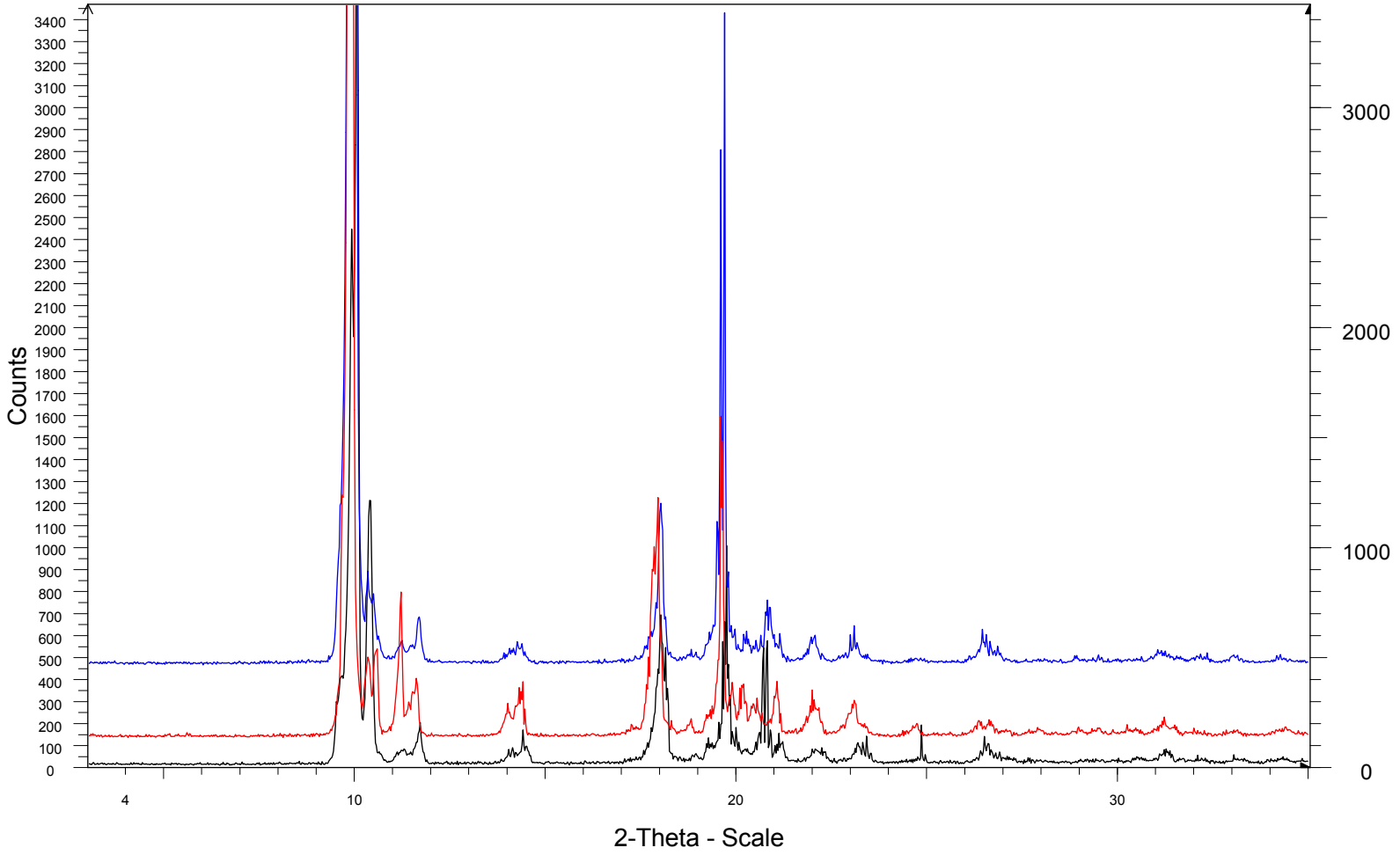
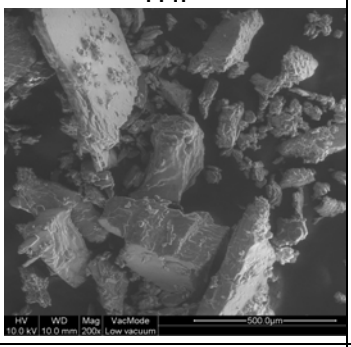
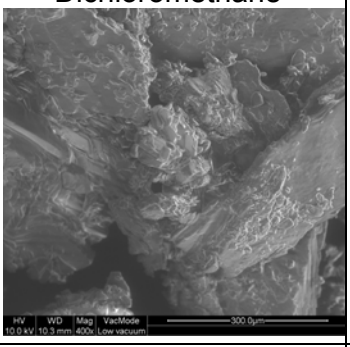
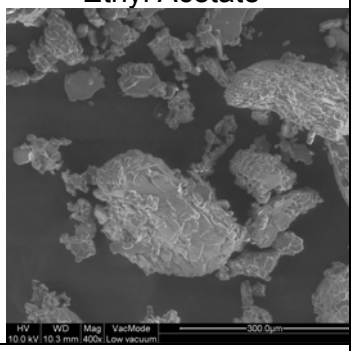
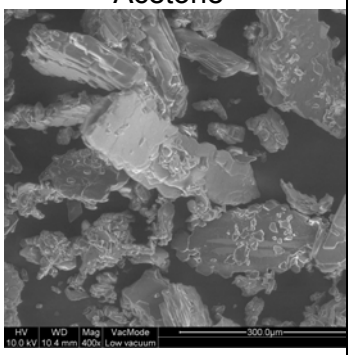
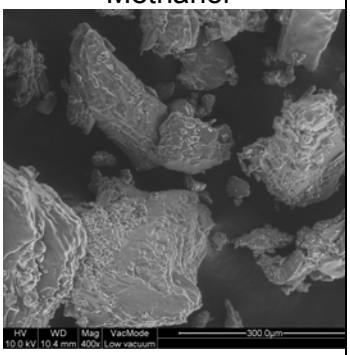
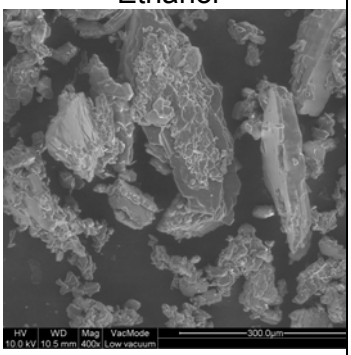
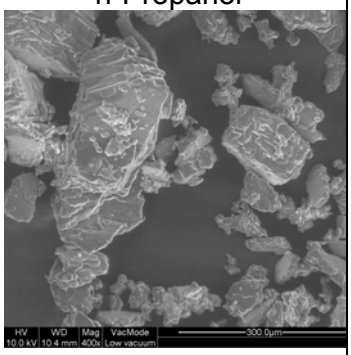
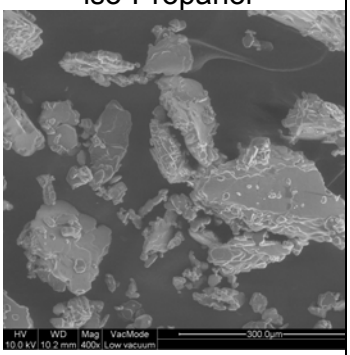
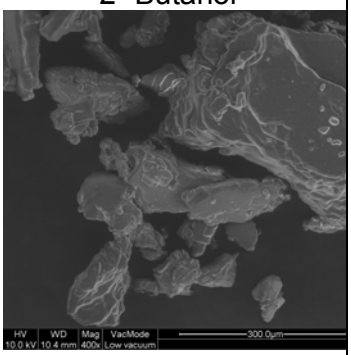
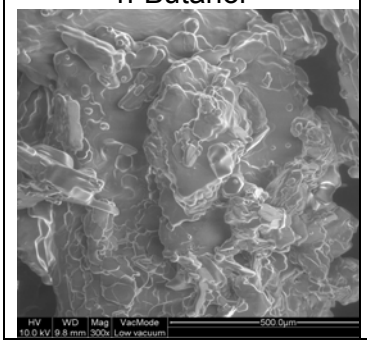
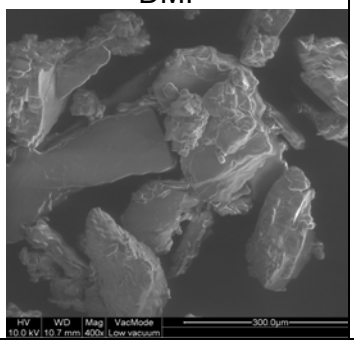
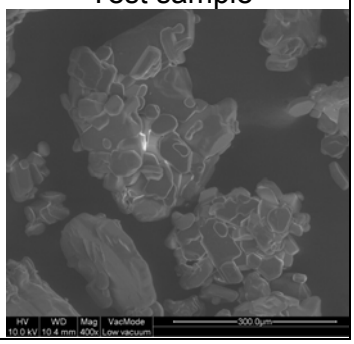


Figure 3.8 Overlay of the XRPD patterns of artemether **test sample** and recrystallised from **n-butanol** and **DMF**.

Table 3.4 Examples of SEM photomicrographs of the recrystallisation products of artemether

THF	Dichloromethane	Ethyl Acetate
		
Acetone	Methanol	Ethanol
		
n-Propanol	iso-Propanol	2- Butanol
		
n-Butanol	DMF	Test sample
		

The DSC results obtained from crystals, prepared by recrystallisation of artemether in a variety of organic solvents, confirmed only one polymorphic form, which corresponded to that of the test sample. Shek e*t al.* (2001) described a stable, high melting point (not provided) form, with crystal parameters of a = 18,158(5) b = 10.070(3) c = 19.360(5)Å β = 112.51(1)°, V = 3270Å^3. Unfortunately, no other physico-chemical data, but the single crystal, X-ray data was presented to compare their form with the crystals obtained during this study.

During this study, only one endothermic event during melting was observed. No evidence of a metastable phase that underwent a phase transformation into a more stable form was observed. Therefore, it could be concluded that the form obtained during this recrystallisation study, was the thermodynamically stable form. The melting points obtained from the various recrystallisations (87 – 90°C) compared well with those published in literature, i.e. 86 – 90°C (Galichet *et al*., 2004:648; O'Neil *et al*., 2006:133; Ph. Int., 2008).

The only differences observed during this preliminary polymorph screening study were the minor differences in the XRPD patterns, obtained for the DMF and n-butanol products, which could have been due to particle size differences and preferential orientation. Observed intensities can differ considerably amongst different samples from the same substance (Byrn *et al*., 1999:63).

3.5 Pharmaceutical and pharmacological background of lumefantrine

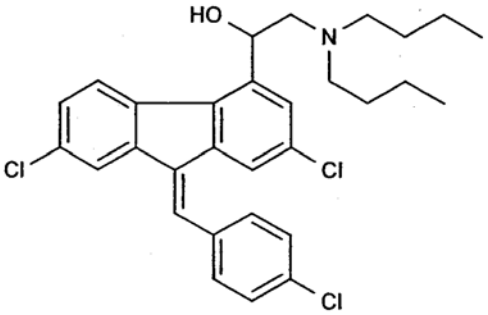
3.5.1 Description and nomenclature

Lumefantrine is a yellow, crystalline powder.

Empirical formula: $C_{30}H_{32}Cl_3NO$

Molecular weight: 528.9

Structural formula:



Chemical name: 2-Dibutylamino-1-[2,7-dichloro-9-94-chlorobenzylidene)-9H-fluoren-4-yl]-ethanol (racemate); CAS Reg. No. 82186-77-4

Nonproprietary name: Lumefantrine, Benflumetol.

3.5.2 Mechanism of action and pharmacology

Lumefantrine is a racemic, fluorene derivative. It resembles the class two blood schizonticides, such as quinine, halofantrine and mefloquine (Ezzet *et al*., 2000:697). The mechanism of action for lumefantrine is not yet fully known, but it seems to be similar to those of halofantrine and the other quinolones (Shapiro & Goldberg, 2010). According to biochemical studies, lumefantrine is trapped in the intra-erythrocytic parasite, followed by binding to toxic haemin (FP9), produced during the course of haemoglobin digestion. This binding prevents the polymerisation of haemin to non-toxic malaria pigment. Lumefantrine therefore also shares its mechanism of action with the class one blood schizontocides (4-aminoquinolines) (Rush *et al*., 2009:2564).

Lumefantrine is absorbed and cleared slower than artemether, which makes it ideal for the elimination of residual parasites, not yet removed by artemether or DHA. Lumefantrine has an elimination half-life of about 3 - 4 days in patients with *Plasmodium falciparum* malaria and 6 days in healthy volunteers. A high-fat meal increased the bioavailability of lumefantrine sixteen-fold, compared to the fasted state, according to the findings of Djimdé and Lefèvre (2009:2).

3.6 Physico-chemical properties of lumefantrine

The lumefantrine test sample used during this study was from Novartis, batch no. C0189, as provided by the WHO.

Lumefantrine is practically insoluble in water, freely soluble in dimethylformaldehyde and ethyl acetate, soluble in dichloromethane, slightly soluble in ethanol and methanol (WHO: testing monograph provided by manufacturer).

The literature only describes one polymorphic form for lumefantrine. The IR, XRPD and DSC data, as described in literature for lumefantrine form I, are presented in Section 3.6.1 (De *et al.*, 2006:1-14).

Saturated solutions of lumefantrine were prepared in glass beakers or polytops, by heating each solvent close to its boiling point, whilst continuously stirring with a magnetic stirrer and slowly adding lumefantrine powder. The polytops or beakers were then sealed with perforated plastic caps or parafilm, allowing the solvent to slowly evaporate at room temperature.

The saturated solutions were prepared in the following organic solvents: acetone, acetonitrile, n-butanol, 2-butanol, chloroform, dichloromethane (DCM), diethylether, dimethylformamide (DMF), ethanol, ethyl acetate, iso-propanol, methanol, n-propanol and toluene (Table 1, Appendix A). The physico-chemical properties of the recrystallised samples are discussed in Sections 3.6.2 – 3.6.4.

In literature, several studies reported significant differences in the physico-chemical properties of various raw materials, obtained from different manufacturers (Liebenberg *et al.,* 1998:485; Liebenberg *et al.,* 1999:1027; Henwood *et al*., 2000:405; Swanepoel *et al*., 2000:1075; Terblanche *et al*., 2000:537). A melting point, ranging between 120°C - 130°C, was reported for lumefantrine (De *et al*., 2006:13). This rather wide range in the melting point prompted testing of three commercial batches of lumefantrine, in order to investigate the physico-chemical properties of lumefantrine from different manufacturers (Section 3.6.6). This aimed at confirming whether the

differences in melting points were due to differences in the raw material, other than polymorphic differences.

3.6.1 Lumefantrine polymorphic form I

Lumefantrine polymorphic form 1 is described in patent WO 2006/117616 A1 (De *et al.,* 2006:1-13). This patent describes characteristic DSC thermograms, with melting points ranging between 120°C - 130°C, as seen in Figure 3.10 (De *et al.,* 2006:13). In Figure 3.9 and Table 3.5, the XRPD pattern and the peak angles of lumefantrine form I can be seen. Figure 3.11 shows the IR spectrum, characteristic to that of lumefantrine form I, as described in literature (De *et al.,* 2006:14). Another publication (Deng *et al.*, 2006:1-8) describes the isomers and enantiomers of lumefantrine and reports the melting point of the lumefantrine racemate as 130°C and those of the enantiomers, with lower solubility, as 149 - 151°C. The racemate, with the lower melting point, shows a conglomerate (mixture of crystals of the individual enantiomers) at a ratio of 1:1.

The specific optical rotation (SOR) of some of the lumefantrine recrystallisation products was determined, one sample had a SOR of -3.2 and a melting point of 142°C whereas all the other samples tested produced results of 0.2 – 0.3 (SR) with melting points in the range of 133 – 140°C.

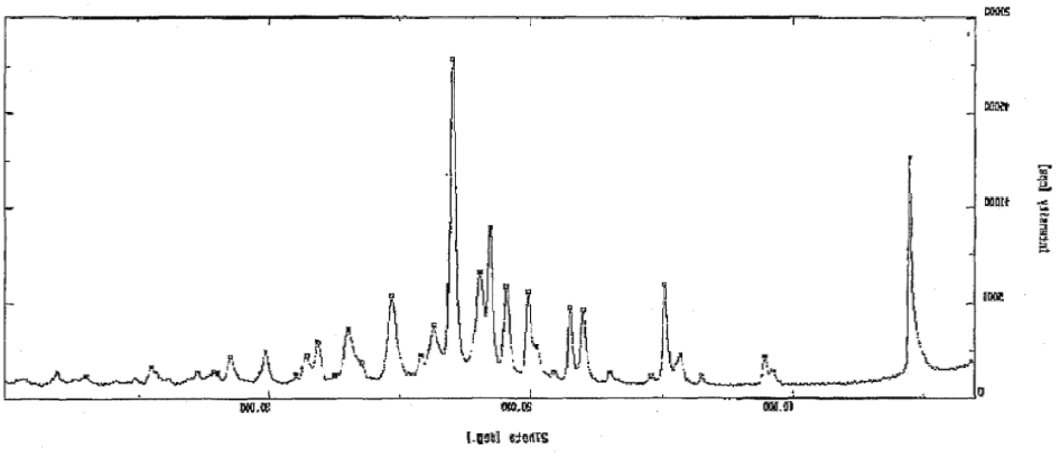


Figure 3.9 XRPD pattern of lumefantrine crystals, similar to form I (De *et al.,* 2006:12).

Table 3.5 Main XRPD peak angles (°2θ) of lumefantrine form I (De *et al.,* 2006:5-6)

Peak angles (°2θ)	Peak angles (°2θ)	Peak angles (°2θ)	Peak angles (°2θ)
5.5	18.5	24.2	31.5
10.8	19.1	25.3	32.0
11.1	19.8	26.5	32.1
13.5	20.1	27.0	32.7
14.3	20.9	27.5	34.5
14.9	21.5	28.2	36.9
15.4	21.9	28.5	38.0
17.0	23.0	29.0	–
18.0	23.7	30.1	–

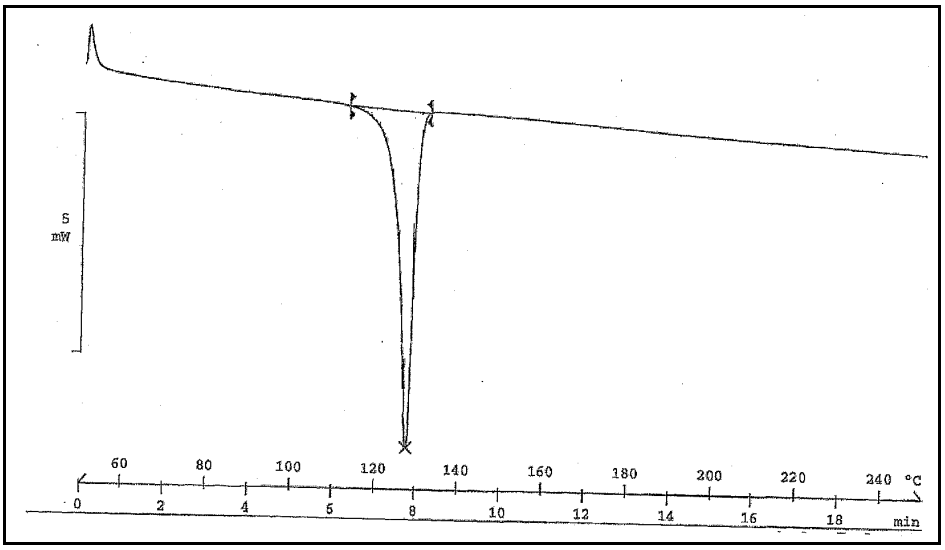


Figure 3.10 DSC trace of lumefantrine form I (De *et al.,* 2006:13).

As was discussed in the literature, form I has a melting point, ranging between 120°C - 130°C. DSC results obtained for the recrystallised samples during this study showed similarities, as well as some differences, compared to form I and are discussed in Section 3.6.4.

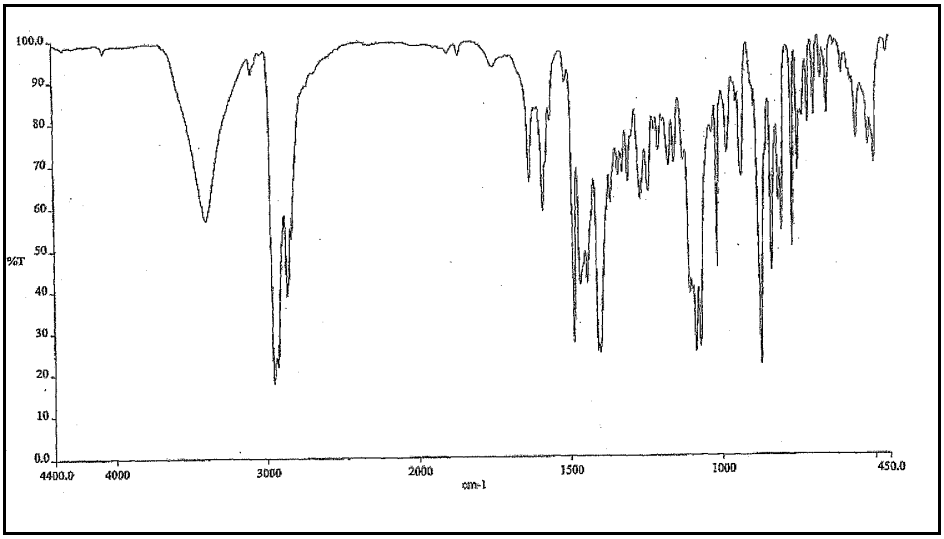


Figure 3.11 IR spectrum of lumefantrine form I (De *et al.,* 2006:14).

3.6.2 Infrared spectra of the recrystallised lumefantrine

The IR spectra of all the recrystallisation products (Figure 3.12) were similar to that reported for form I (Figure 3.11). The main IR absorptions for the recrystallisation product from acetone, are listed in Table 3.6 as an example.

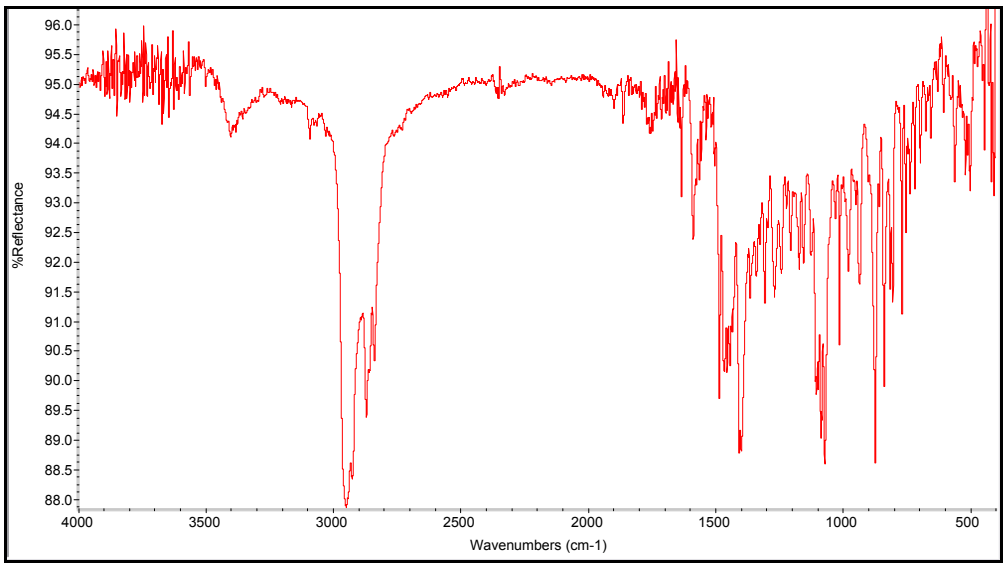


Figure 3.12 IR spectrum of lumefantrine recrystallised from acetone.

Table 3.6 Main absorptions in the IR spectrum of lumefantrine recrystallised from acetone

Main absorptions	Wavenumbers (cm^{-1})	Main absorptions	Wavenumbers (cm^{-1})
1	718	**24**	1242
2	736	**25**	1269
3	753	**26**	1308
4	770	**27**	1341
5	806	**28**	1366
6	815	**29**	1399
7	839	**30**	1408
8	859	**31**	1443
9	874	**32**	1455
10	934	**33**	1465
11	951	**34**	1487
12	980	**35**	1504
13	1014	**36**	1539
14	1030	**37**	1558
15	1071	**38**	1564
16	1086	**39**	1588
17	1098	**40**	1634
18	1105	**41**	1760
19	1127	**42**	2840
20	1156	**43**	2871
21	1173	**44**	2951
22	1206	**45**	3092
23	1222	**46**	3402

3.6.3 X-ray powder diffraction (XRPD) of the recrystallised lumefantrine

The XRPD results (Figure 3.13 and Table 3.7) obtained from all the recrystallised samples were found to be concordant to that of form I (Figure 3.9).

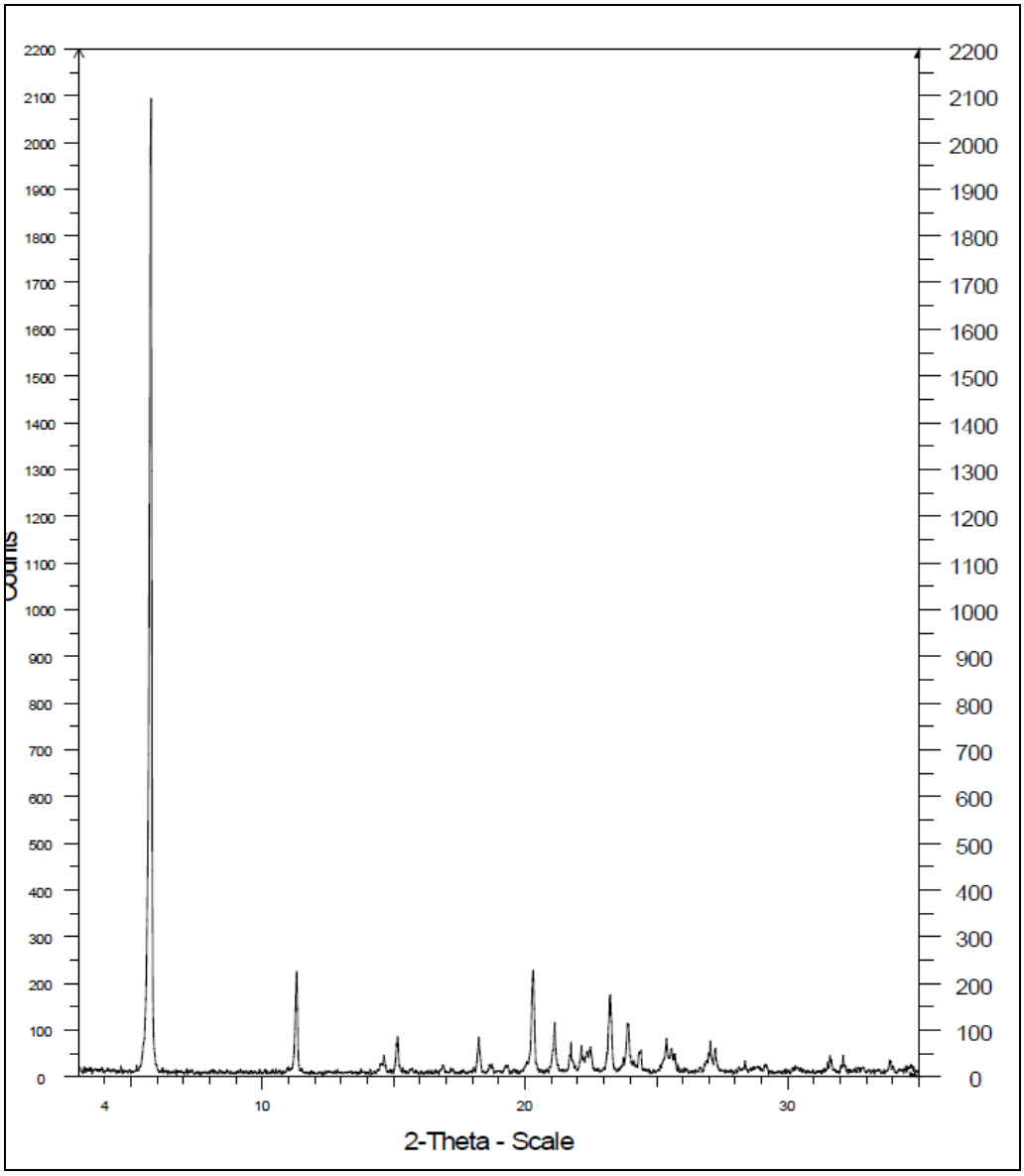


Figure 3.13 XRPD pattern of lumefantrine recrystallised from acetone.

Table 3.7 Peak intensity ratios (I/Io) at main XRPD peak angles (°2θ) of lumefantrine recrystallised from acetone

Peak angles (°2θ)	Relative intensities (I/Io)
5.7	100
11.3	11
14.6	2
15.1	4
16.8	1
18.2	4
18.7	1
19.3	1
20.3	11
21.1	5
21.7	3
22.2	3
22.5	3
23.2	8
23.9	5
24.4	3
25.4	4
25.6	3
27.1	4
27.3	3
29.2	1
31.7	2
32.2	2
34.0	1
34.7	1
35.3	2

3.6.4 Differential scanning calorimetric (DSC) results of the recrystallised lumefantrine

Figure 3.14 illustrates an example of a DSC trace obtained from an acetone recrystallisation. The melting points of the lumefantrine recrystallisation products were between 124 – 143 °C. This variation in melting points is discussed in Section 3.6.5.

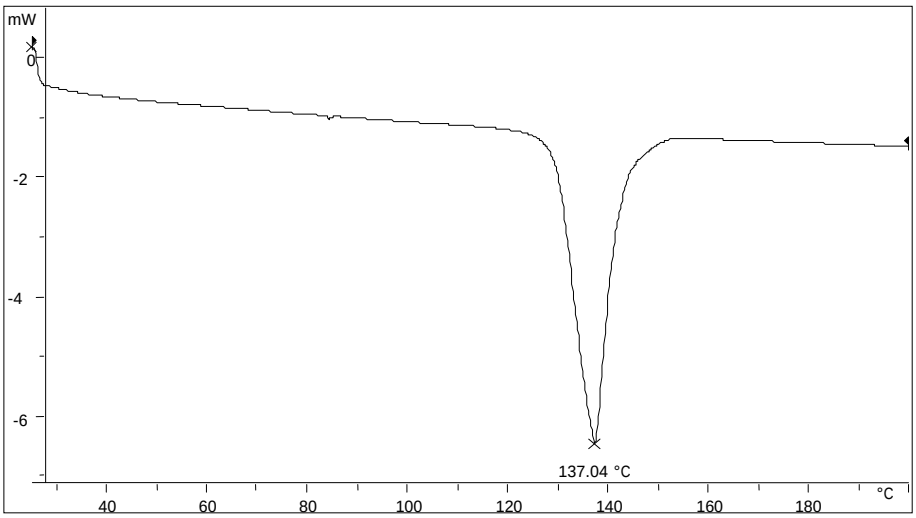


Figure 3.14 DSC trace of lumefantrine recrystallised from acetone.

3.6.5 Influence of crystal habit, size and preferred orientation on physico-chemical properties of lumefantrine

When the shapes of crystallites are anisotropic (e.g. platelet- or needle-like), it may result in distinctly non-random, crystalline orientation, due to the natural preferences in packing of these particles. This non-random, particle orientation, called *preferred orientation*, may cause considerable distortions of the scattered intensity of the reflections (Pecharsky & Zavalij, 2005:196).

In Figures 3.15 and 3.18, peaks A, B and C clearly illustrate preferred orientation effects, due to the differences in particle sizes with regards to XRPD, while the DSC traces in Figures 3.16 and 3.17 show the effects of varying particle sizes on melting point. The influence of rotation of XRPD samples on preferred orientation was also compared to that of non-rotating samples (Roberts *et al.*, 2002:1149). For the sake of this experiment, the X-ray data was collected where the powder sample holder was

rotated, whilst a second measurement on the same sample was taken where the sample holder was not rotated.

With this experiment, no significant difference was observed between the rotating sample and the non-rotating sample. The differences in particle sizes, however, resulted in some differences in the diffractograms (Tables 3.8 and 3.9). The IR spectra of the different particle size samples did not show any differences and were concordant to that of form I.

Table 3.8 Peak intensity (I) at main XRPD peak angles (°2θ) of lumefantrine recrystallised from chloroform

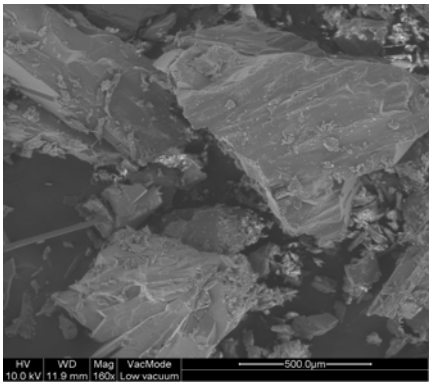
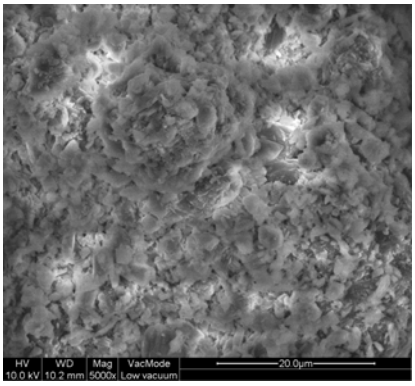
Sample from: Chloroform	Position (°2θ)	Intensity count (I)
PEAK A:		
Crystals rotating	6.0	198
Crystals not rotating	6.0	242
Finely ground sample rotating	5.7	299
Finely ground sample not rotating	5.7	274
PEAK B:		
Crystals rotating	11.6	841
Crystals not rotating	11.6	815
Finely ground sample rotating	11.3	37
Finely ground sample not rotating	11.2	36
PEAK C:		
Crystals rotating	25.8	128
Crystals not rotating	25.7	142
Finely ground sample rotating	25.6	68
Finely ground sample not rotating	25.5	57
Coarse crystals	**Finely ground sample**	
 (Mag. 160X) Particle size $D_{(0.5)}$: 30.03 µm	 (Mag. 5000x) Particle size $D_{(0.5)}$: 19.60 µm (Possible agglomeration of powder)	

Table 3.9 Peak intensity (I) at main XRPD peak angles (°2θ) of lumefantrine recrystallised from methanol

Sample from: Methanol	Position (°2θ)	Intensity counts (I)
PEAK A:		
Crystals rotating	5.9	84
	6.1	125
Crystals not rotating	5.9	94
	6.1	103
Finely ground sample rotating	5.6	424
Finely ground sample not rotating	5.6	377
PEAK B:		
Crystals rotating	18.8	127
Crystals not rotating	18.8	114
Finely ground sample rotating	18.1	46
	18.6	63
Finely ground sample not rotating	18.1	58
	18.6	73
PEAK C:		
Crystals rotating	23.5	260
Crystals not rotating	23.5	327
Finely ground sample rotating	23.8	53
Finely ground sample not rotating	23.1	184
Particle size of coarse crystals $D_{(0.5)}$: 32.30 µm Particle size of finely ground sample $D_{(0.5)}$: 18.02 µm		

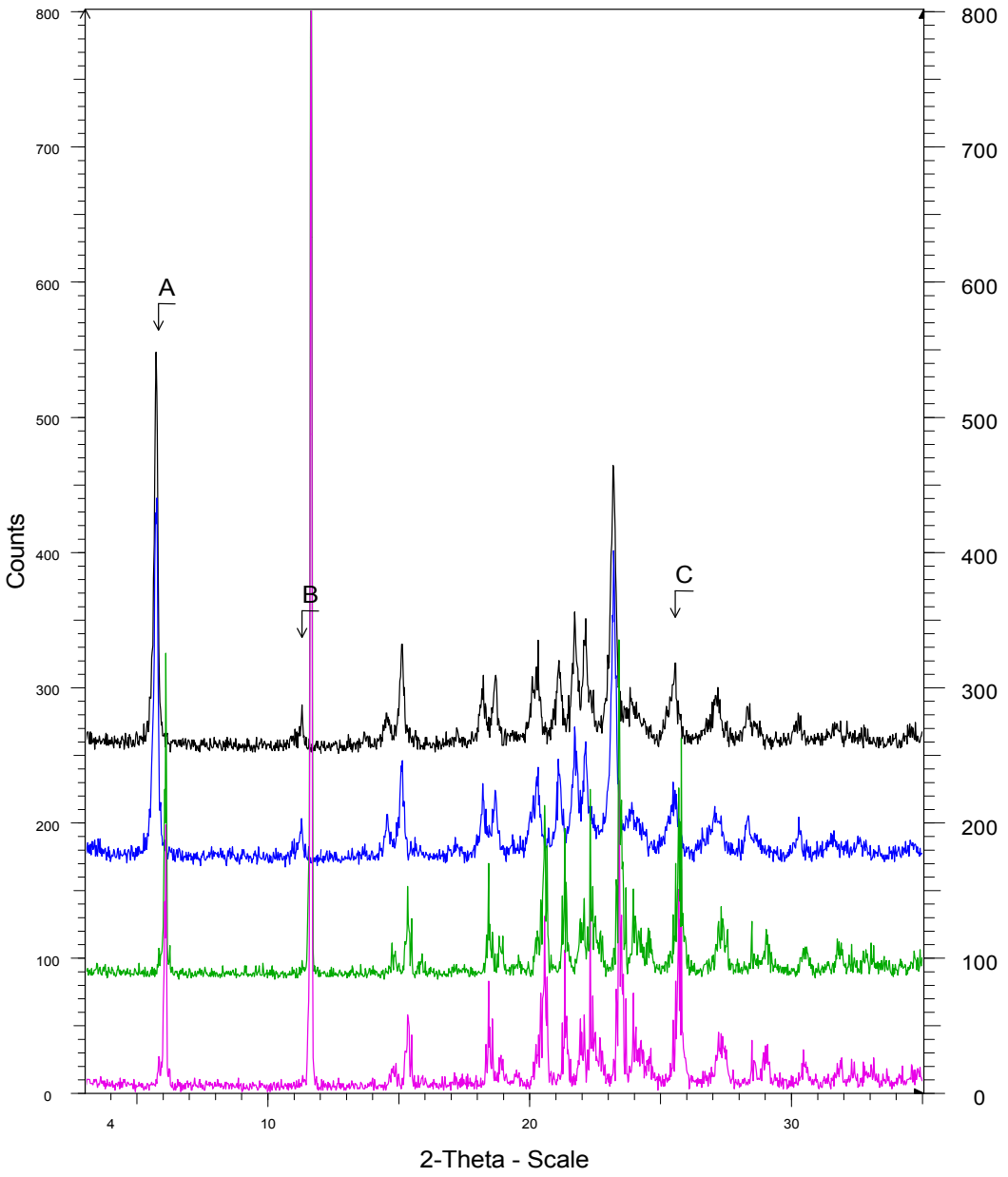


Figure 3.15 Overlay of the XRPD patterns of lumefantrine crystals (recrystallised from chloroform), showing the influence of preferred orientation: **finely ground sample rotating**, **finely ground sample not rotating**, **coarse crystals not rotating** and **coarse crystals rotating**.

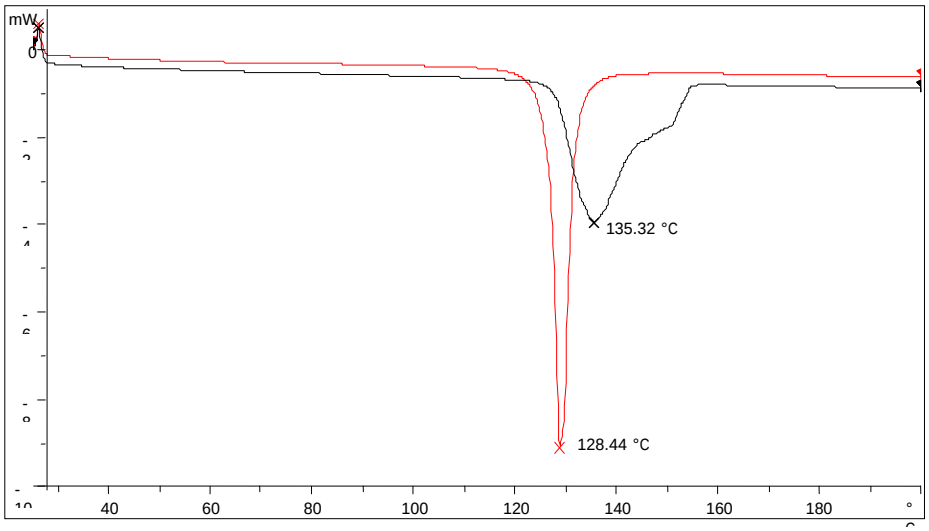


Figure 3.16 Overlay of DSC traces of **lumefantrine crystals** (recrystallised from chloroform) and the **finely ground sample** thereof.

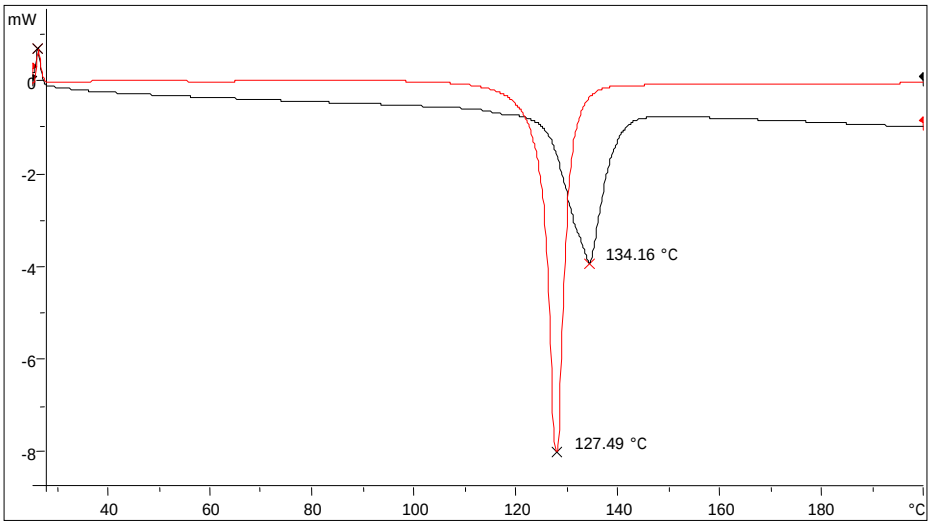


Figure 3.17 Overlay of DSC traces of **lumefantrine crystals** (recrystallised from methanol) and the **finely ground sample** thereof.

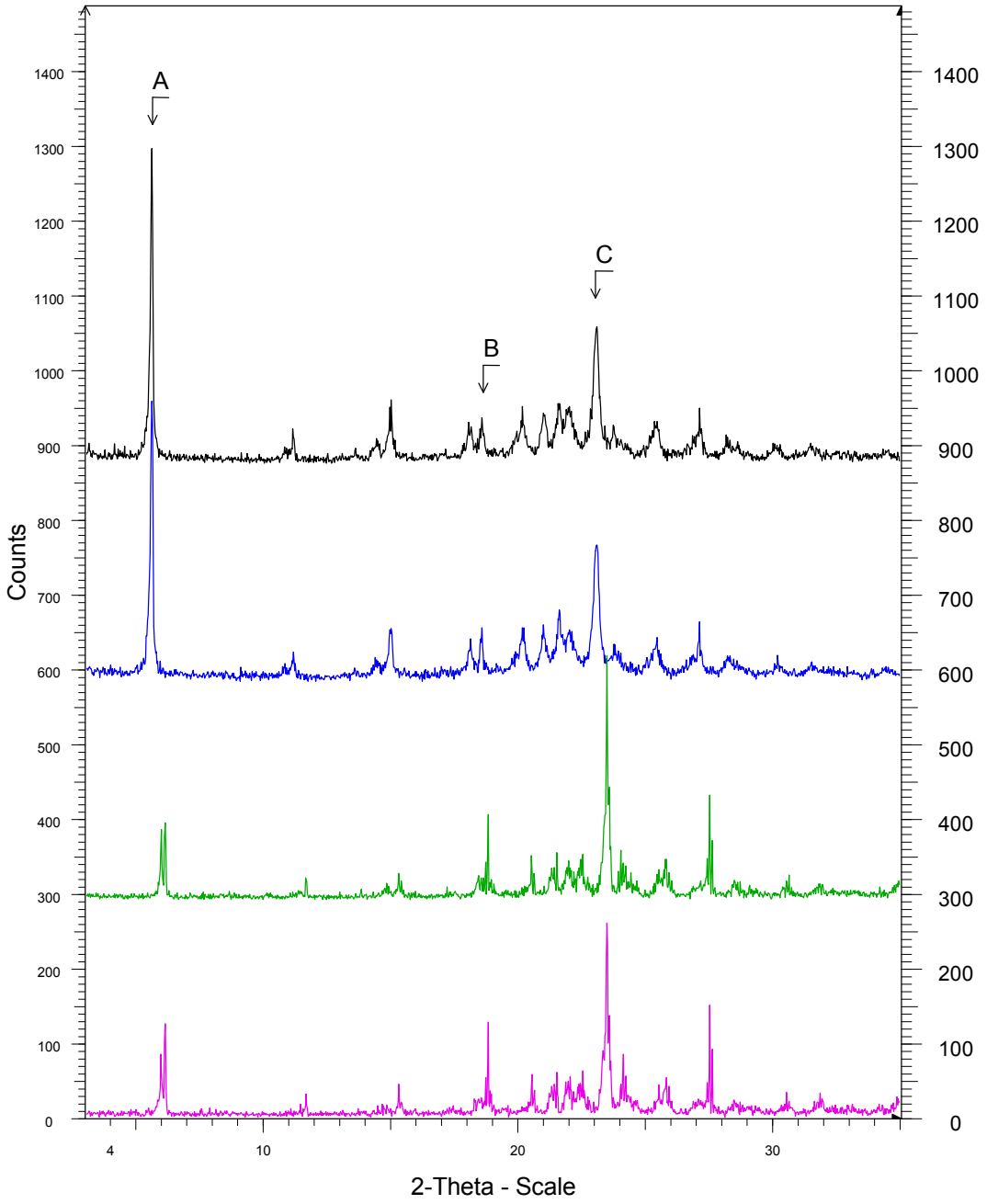


Figure 3.18 Overlay of the XRPD patterns of lumefantrine crystals, showing the influence of preferred orientation on crystals recrystallised from methanol: **finely ground sample rotating**, **finely ground sample not rotating**, **coarse crystals not rotating** and **coarse crystals rotating**.

In a study on ketoconazole, it was found that different solvents used for crystallisation, produced crystals of the same polymorphic form, but with different habits and melting points (Viseras *et al.,* 1995:145-151). In this study, lumefantrine crystals, produced by

most solvents (Table 1, Attachment A), showed a similar scenario, although the different lumefantrine recrystallisation products were poorly defined aggregates, dissimilar, and with no discernible trend in crystal habit. These differences in crystal habit of lumefantrine were illustrated by the SEM and TM photos (Tables 3.10 and 3.11). The DSC traces in Figures 3.16 and 3.17 confirmed a difference in melting point due to particle size, with an increase in melting point with an increase in particle size.

Because of this phenomenon, is it advisable not to characterise and identify lumefantrine polymorphic forms, based on their melting points.

Table 3.10 SEM photomicrographs and melting points of lumefantrine crystals recrystallised from different solvents

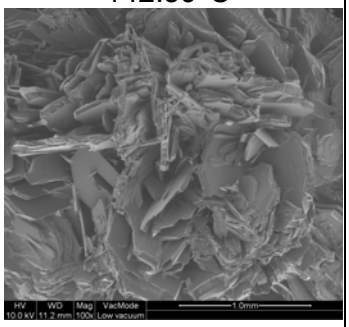
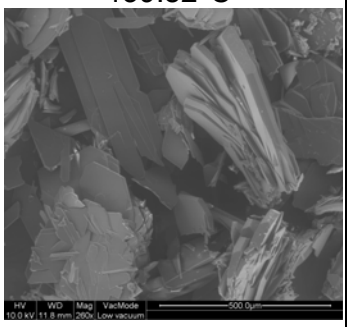
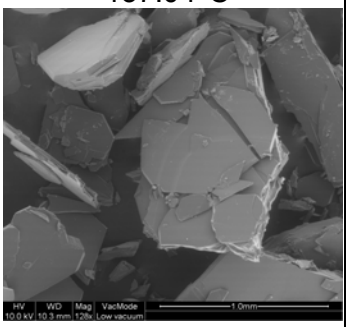
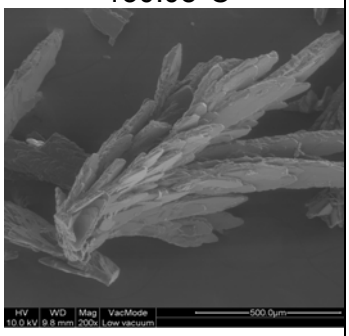
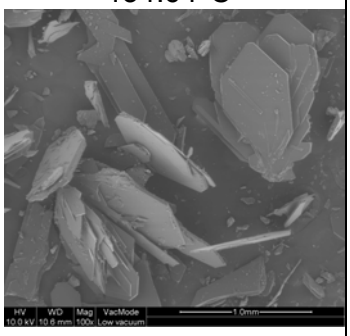
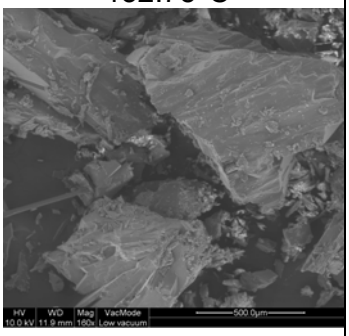
Dichloromethane 142.59°C	Ethyl acetate 139.82°C	Acetone 137.04°C
		
Ethanol 130.08°C	Diethylether 134.64°C	Chloroform 132.75°C
		

Table 3.10 **(continued)** SEM photomicrographs and melting points of lumefantrine crystals recrystallised from different solvents

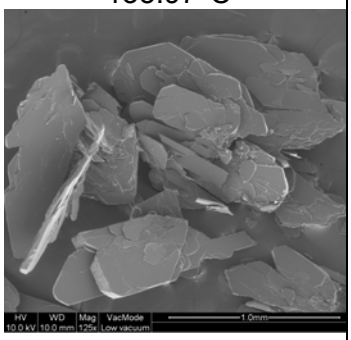
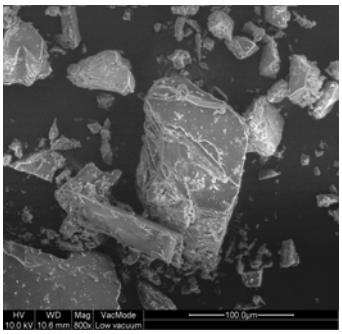
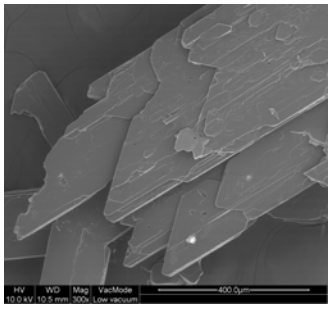
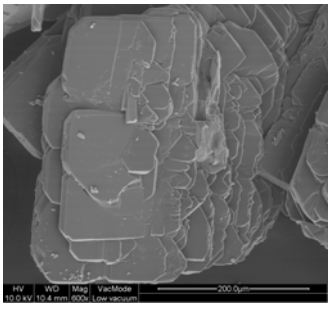
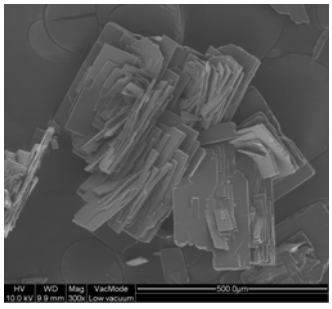
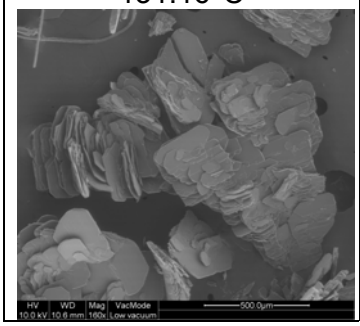
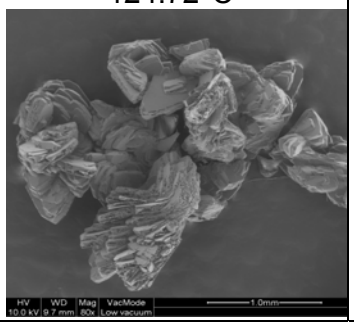
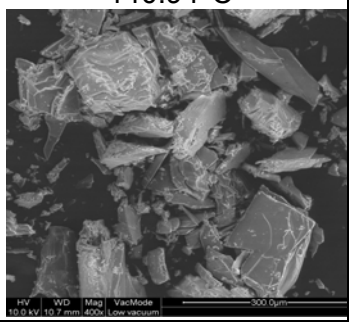
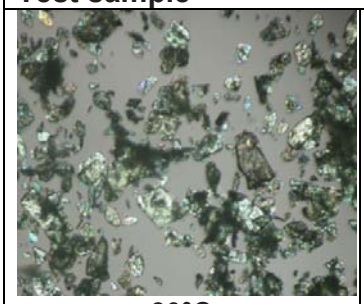
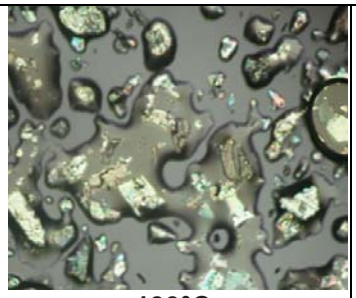
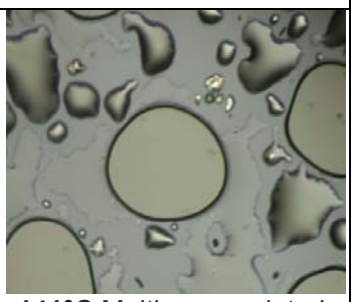
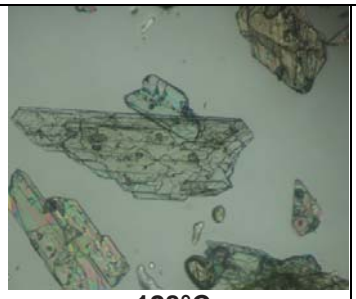
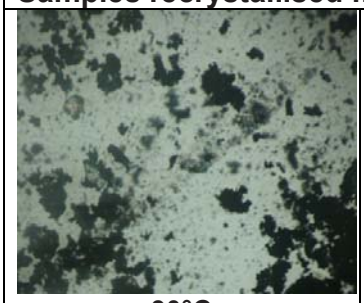
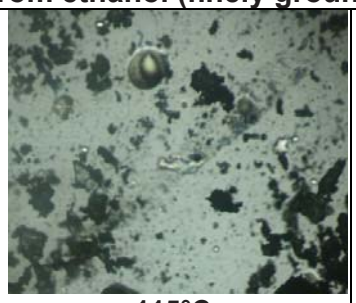
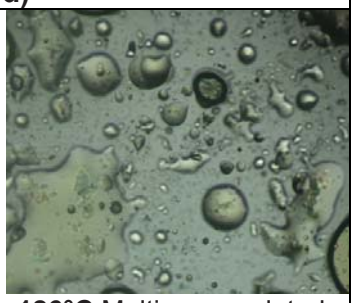
Propan-1-ol 133.97°C	2-Butanol 133.91°C	DMF 133.25°C
		
Acetonitrile 133.67°C	Methanol 133.97°C	1-Butanol 133.97°C
		
Propan-2-ol 131.16°C	DMSO 124.72°C	Toluene 140.94°C
		

Table 3.11 Thermomicroscopic results for lumefantrine test sample and crystals recrystallised from different solvents

Test sample		
		
30°C	**133°C**	**141°C** Melting completed
Samples recrystallised from acetonitrile		
		
30°C	**128°C**	**132°C** Melting completed
Samples recrystallised from ethanol		
		
30°C	**91°C**	**126°C** Melting completed at 131°C
Samples recrystallised from ethanol (finely ground)		
		
30°C	**115°C**	**126°C** Melting completed

3.6.6 Investigation of commercial lumefantrine batches

Three batches of lumefantrine raw material (Table 3.12) were obtained from different suppliers and subjected to IR (Figure 3.19), XRPD (Figure 3.22), DSC (Figure 3.20) and particle size (Table 3.13) testing. In addition, photomicroghraphs were taken to compare the crystal habits (Table 3.14).

Table 3.12 Commercial lumefantrine materials procured from different suppliers

Sample code	Batch number	Manufacturing company
RM 1	C0189	Novartis
RM 2	070701	Tianjin Hi-tesion Bio & Chem Co., Ltd.
RM 3	090306	IFF, Iffect Chemphar (HK) Company Limited

The IR spectra of the three batches of commercial lumefantrine materials tested, (Figure 3.19) were concordant to that of lumefantrine form I.

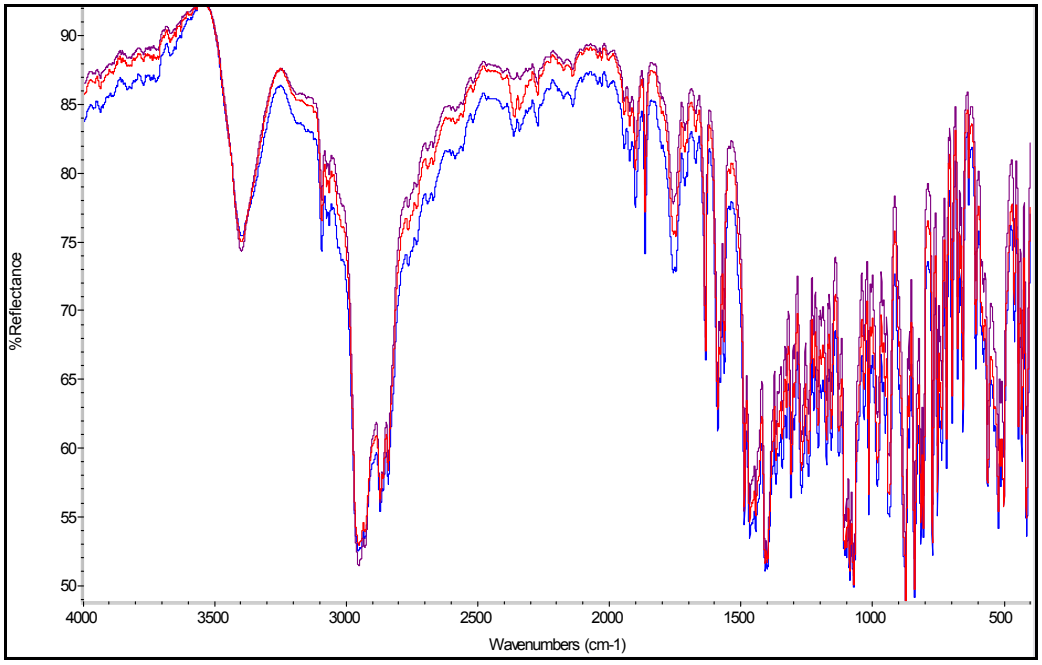


Figure 3.19 IR spectra of lumefantrine samples **RM1**, **RM2** and **RM3**.

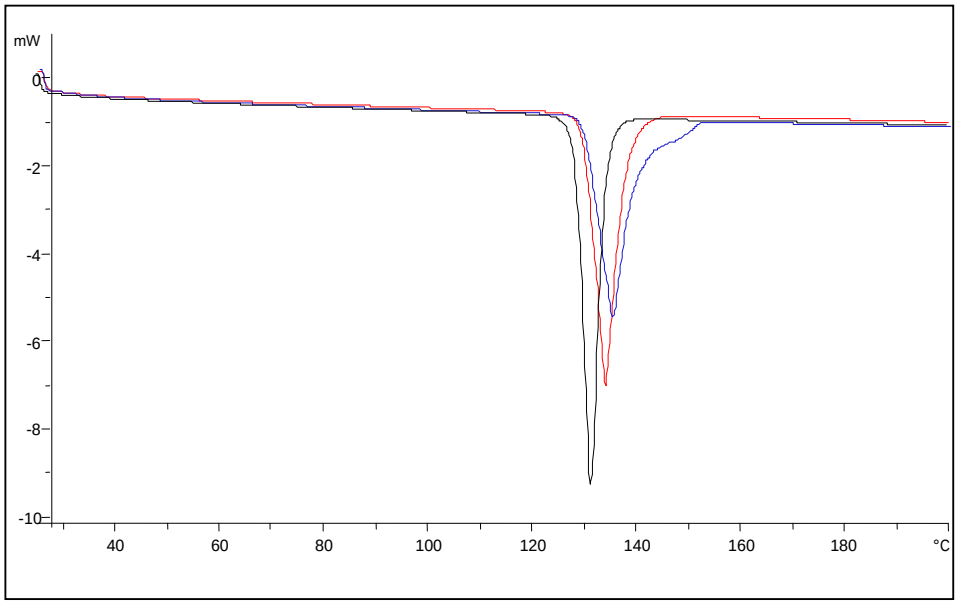


Figure 3.20 DSC traces of lumefantrine samples **RM1** (131.1°C), **RM2** (134.6°C) and **RM3** (133.7°C).

Although the differences in particle size were insignificant, the sample with the lowest melting point (RM1), also had the smallest particle size, as expected (Xue *et al.,* 2001:388-390). The melting points of the commercial materials being tested showed that the larger the particle size, the higher the melting point: RM1<RM3<RM2 (Table 3.13). The relationship between particle size and melting point is illustrated in Figure 3.21.

Table 3.13 The melting points and volume distribution ($D_{(0.9)}$) of the three commercial lumefantrine batches

Raw material	Particle size $D_{(0.9)}$ (µm)	Melting point (°C)
RM1	107.4	131.1
RM2	144.7	134.6
RM3	130.1	133.7

Figure 3.22 displays an overlay of the XRPD patterns of the three commercial batches of lumefantrine being tested. The particle size and habit differences between these three batches did not differ significantly enough to have an effect on the XRPD patterns. The XRPD patterns were also identical to that of the reported form 1 (De *et al.,* 2006:12).

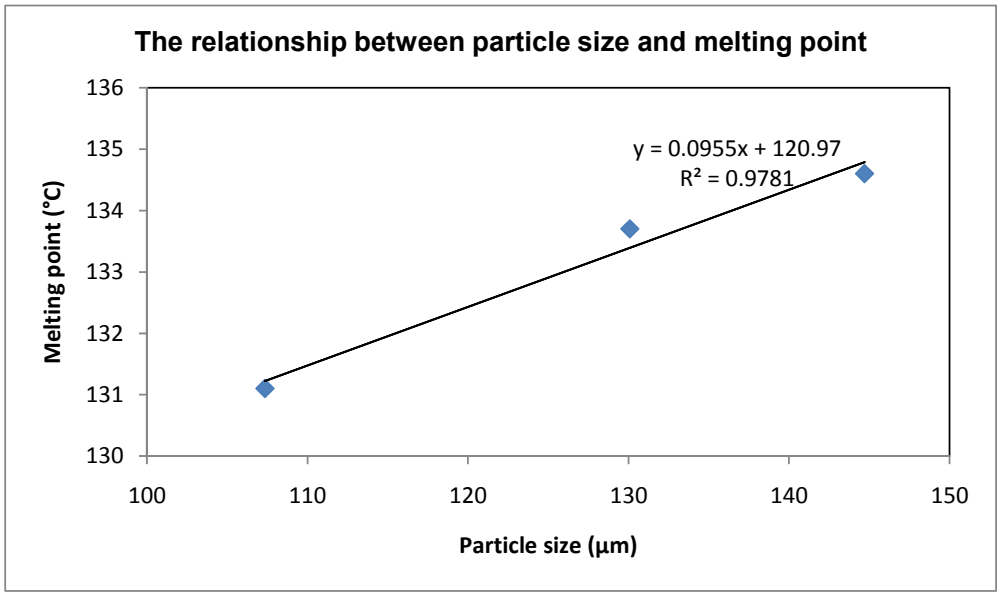
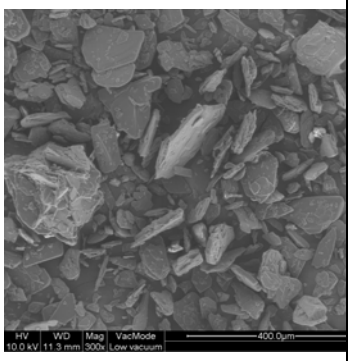
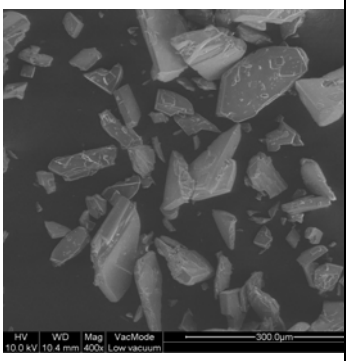
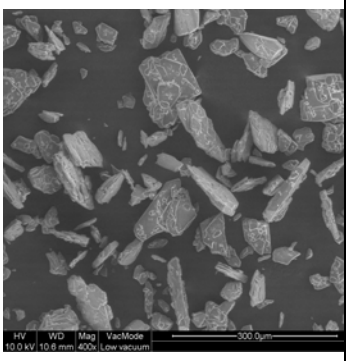


Figure 3.21 Relationship between particle size and melting point of lumefantrine.

Table 3.14 SEM photomicrographs of lumefantrine samples RM1, RM2 and RM3

RM 1	RM2	RM 3
		

The SEM photomicrographs (Table 3.14) illustrate the habits and particle sizes of lumefantrine samples RM1 (mag 300x), RM2 (mag 400x) and RM3 (mag 400x).

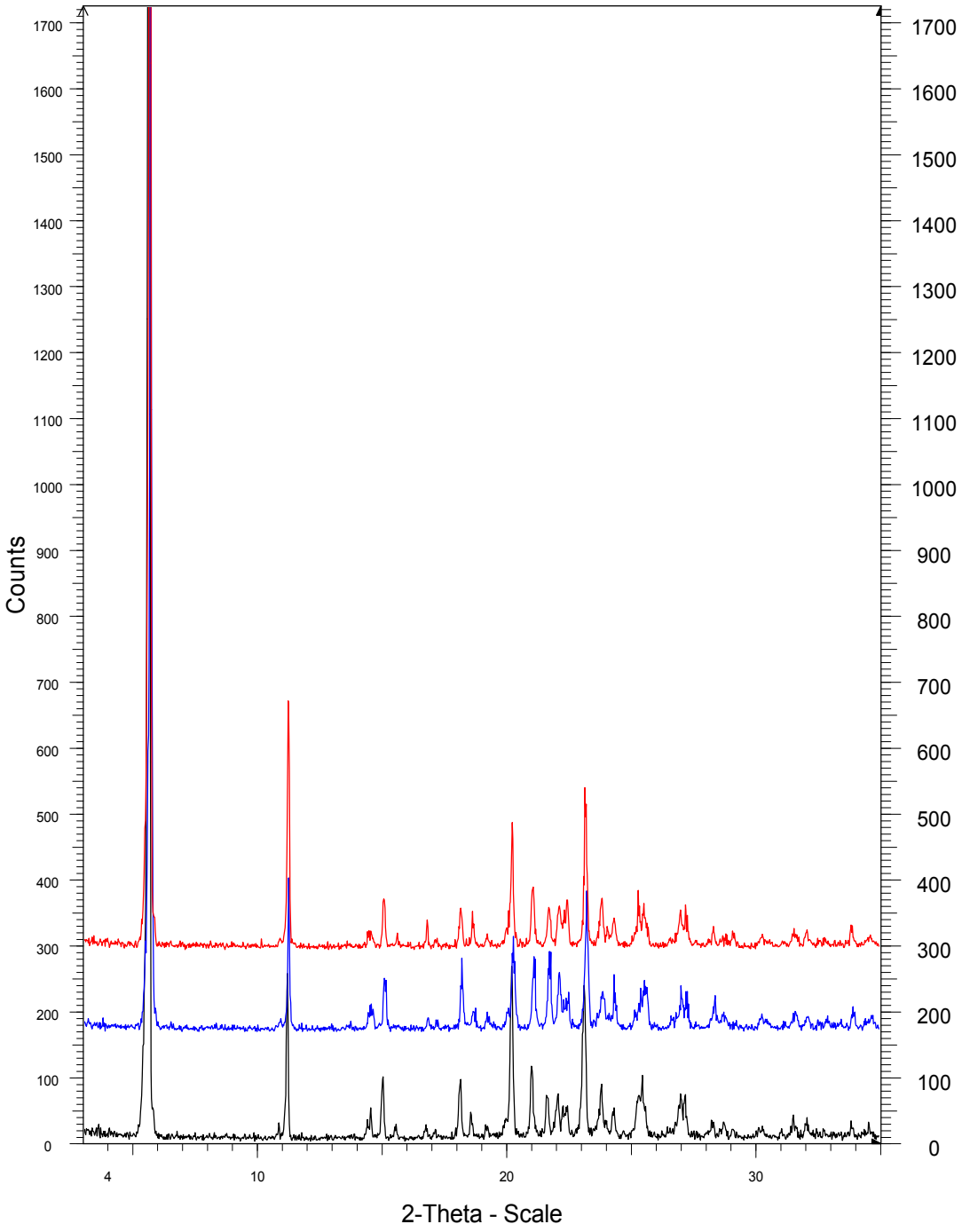


Figure 3.22 XRPD patterns of lumefantrine samples **RM1**, **RM2** and **RM3**.

3.7 Conclusion

In the development of monographs for artemether and lumefantrine raw materials and for artemether/lumefantrine combination products, an important part of the investigation process of these APIs was to determine the possibility of polymorphism. The results obtained during this investigation were concluded as follows:

When artemether was screened for probable polymorphic forms, all the physico-chemical results of the different recrystallisation products proved to be concordant to those of the raw material. Based on the melting points obtained, only one endothermic event was observed during melting. No metastable phase that underwent a phase transformation into a more stable form was observed. It could therefore be concluded that the form obtained during this recrystallisation study was a stable form.

Polymorphic screening of lumefantrine revealed no new polymorphic forms. It was, however, found that the XRPD patterns (Section 3.6.5) of lumefantrine crystals (raw material, as well as those produced through recrystallisation from different solvents) were influenced by preferred orientation. Furthermore, melting points between 124 – 143°C were reported. These differences in melting point correlated well with differences observed in particle size and/or morphology.

The DSC traces confirmed a difference in melting point due to particle size, with an increase in melting point with an increase in particle size. Manufacturers of lumefantrine should keep in mind that the melting point could be influenced by crystal habit and particle size, and is it advisable not to characterise and identify lumefantrine polymorphic forms, based on their melting points.

CHAPTER 4

Monograph development for the lumefantrine API

4.1 Introduction

The importance for the development of monographs for antimalarial medicines, as well as the processes and the guidelines used in the development thereof, were discussed in Chapters 1 and 2. The WHO had identified the need for the inclusion of a monograph in the Ph.Int. for the lumefantrine API to support the proposed monographs for artemether/lumefantrine tablets and artemether/lumefantrine oral suspension (powder for oral suspension). A monograph for the artemether API already exists in the current edition of the Ph.Int. (2008).

The development and validation of quality control (QC) tests and acceptance criteria for lumefantrine are discussed in this chapter. The WHO provided the manufacturer specifications for lumefantrine, as well as a primary standard of the API and standard materials of its related substances for use during this study. Commercial samples of lumefantrine were also included in this study. The solid-state studies performed on lumefantrine (Chapter 3) provided additional information, necessary for the lumefantrine monograph.

During the development processes of the lumefantrine monograph, draft specifications had been sent to the expert panel and specialists of the WHO for peer reviewing. Comments received had been considered and included in the specifications, where appropriate.

The lumefantrine monograph (as adopted by the WHO Expert Committee on Specifications for Pharmaceutical Preparations, held in Geneva during October 2007 for inclusion in the Ph.Int., second supplement) is included in Appendix B. The adopted monograph was published in July 2008 on the WHO website at http://www.who.int/medicines/publications/pharmacopoeia/Lumef_monoFINALQAS06_186_July08.pdf.

4.2 Specifications developed for the lumefantrine API monograph

The components of API monographs in the Ph.Int. (2008), as listed in Chapter 2 (Table 2.1), were used as a guideline for selecting the methods for inclusion in the development of the lumefantrine API monograph.

The general information selected included the name, structure, molecular formula, relative molecular mass, chemical name, synonyms, description, solubility, category, storage and additional information, i.e. melting point. No specific polymorphic form for lumefantrine was indicated under the additional information, due to the absence of polymorphic forms in the polymorphic screening of lumefantrine API, as reported in Chapter 3.

The requirements for the monograph included the following (Table 2.1):

- Definition;
- Identity tests (UV, TLC and IR);
- Heavy metals;
- Sulfated ash;
- Loss on drying;
- Related substances (HPLC and TLC); and
- Assay (titration).

4.2.1 Definition

The limits for the assay of the lumefantrine API are: Lumefantrine contains not less than 98.5% and not more than 101.0% of $C_{30}H_{32}Cl_3NO$, calculated with reference to the dried substance.

- These tight proposed limits were in line with the Ph.Int., where titrations were the selected assay method. Examples from the Ph.Int. included abacavir sulfate (99.0% - 101.0%), indinavir sulfate (98.5% - 101.0%), ritonavir (98.5% - 101.0%) and the antimalarial APIs, mefloquine hydrochloride, pyrimethamine, quinine sulfate and sulfadoxine (all having limits of 99.0% - 101.0%).

- These assay limits are normally asymmetrical (98.5% to 101.0%), when one or more of the related substances do not contribute to the titration, like lumefantrine's impurities, B and C (for their structures, see Figure 4.3), as they

contain no basic atom(s) to react with perchloric acid during titration (refer to section 4.2.7 for the assay method).

4.2.2 Identification

Identity tests are used to verify the identity of APIs, with an infrared (IR) spectrum normally accepted as the primary method for identification. In the instance where an IR spectrophotometer is unavailable, further identification tests collectively may be accepted as sufficient (Ph.Int., 2008).

For the monograph of lumefantrine API, three methods for identification were developed, allowing a choice of either a combination of two of these tests, or one:

- A thin layer chromatography (TLC) and an ultraviolet-visual (UV-VIS) spectrophotometric method in combination; or
- Infrared spectroscopy as an impartial test, due to its high specificity (ICH Q6A, 1999:9).

4.2.2.1 Thin layer chromatography (TLC)

The TLC method from a manufacturer for identification of the APIs in artemether/lumefantrine FDC tablets, as supplied by the WHO, was used as reference in developing a TLC method for lumefantrine API (Table 4.1). The method finally developed for inclusion in the lumefantrine monograph is summarised in Table 4.1.

General procedure for TLC testing

Whenever TLC testing is applied for identification purposes, or for controlling of related substances in this manuscript, the following general procedure, as described in section *1.14.1 Thin-layer chromatography* of the Ph.Int. (2008), was followed, using commercially available, pre-coated TLC plates as stationary phase.

> *"Unless otherwise specified in the monograph, work under saturated chamber conditions. To achieve such conditions, line the chromatographic chamber with filter-paper and pour into the chamber a sufficient quantity of the mobile phase to saturate the filter-paper and form a layer about 5 mm deep. Close the chamber and allow to stand for at least 1 hour at room temperature.*
>
> *All operations during which the plate is exposed to the air should preferably be carried out at a relative humidity of 50-60%. Apply the volume of the solution as specified in the monograph as a compact spot, preferably not more than 4 mm in diameter. Application may be made using a micropipette, a syringe, or other*

suitable means. The spot should be placed about 1.5 cm from the lower edge and not less than 2 cm from the vertical sides of the plate. Where more than one chromatogram is run on the same plate, the spots should be placed not less than 1.5 cm apart and form a line parallel with the lower edge of the plate. When the solvent has evaporated, place the plate in the chromatographic chamber, ensuring that the plate is as nearly vertical as possible and that the starting points are above the level of the mobile phase. Close the chamber and maintain it at a constant temperature. Allow the mobile phase to ascend, usually 10-15 cm, remove the plate, mark the position of the solvent front and dry as specified in the monograph."

Table 4.1 Chromatographic conditions of two TLC methods for identification of lumefantrine in artemether/lumefantrine tablets and lumefantrine API

	Method from manufacturer for artemether/lumefantrine tablets	**Developed method for inclusion in lumefantrine monograph**
Layer	Silica gel 60, F 254	Silica gel 60, F 254
Solvent	Water : ethyl acetate : methanol : chloroform (2 : 2 : 10 : 11) (v/v)	Ethyl acetate
Mobile phase	Light petroleum ether (boiling point: 40ºC - 60ºC) : ethyl acetate : glacial acetic acid (40 : 10 : 5) (v/v)	Petroleum ether (BP 40 - 60ºC) : ethyl acetate : glacial acetic acid (40 : 6 : 10) (v/v)
Concentration of standard/test solutions	4.8 mg/ml (with respect to lumefantrine)	10 mg/ml
Application volume	20 µl	10 µl
Detection A	UV light at 254 nm	UV light at 254 nm
Detection B	Spray the chromatogram with sulfuric acid in methanol 20% (v/v), heat in a drying oven at 140°C for 10 minutes and assess in daylight.	Expose to iodine vapour until spots appear and assess immediately in daylight.
Detection C	After detection B, assess under UV light at 366 nm.	N/A
Evaluation	Assess the agreement of the spots for lumefantrine in the test and standard solutions with respect to Rf value, colour, and approximate size. Lumefantrine appears as a dark spot on a light fluorescent background in detection B, and as a dark spot on a blue fluorescent background in detection C.	The principal spot with solution A (test solution) corresponds in position, appearance and intensity to that obtained with solution B (standard solution).

Development of a TLC method for lumefantrine API identification

The manufacturer's method used for identification of artemether and lumefantrine in artemether/lumefantrine tablets was adjusted in various ways in order to obtain an uncomplicated, yet effective method for the identification of lumefantrine API.

Firstly, a change in the solvent (Msolv) [water : ethyl acetate : methanol : chloroform (2 : 2 : 10 : 11) (v/v)] of the reference method was investigated. Ethyl acetate was considered as a single solvent, due to the fact that it would exclude the possibility of an error in solvent preparation, and as it would eliminate the use of chloroform, which is discouraged by WHO in QC testing, due to its toxicity (Ph.Int., 2005). Ethyl acetate was found to be an effective solvent. As can be seen in Figure 4.1, the spots obtained when using ethyl acetate as solvent were similar in appearance, intensity and position (Rf) to the spots obtained, using the reference method solvent.

The mobile phase was adjusted (as seen in the development of the TLC method for related substances, section 4.2.6.2), to allow a clear separation of the known related substances. This implied that the method for identification would be the same as that of controlling the related substances. Hence by performing one TLC test, both the related substances and identification tests could be performed in a single method.

In Figure 4.1 the effect of different spot volumes is visible. 10 µl was chosen as a suitable volume for the identification test of lumefantrine, based on the shape, intensity and colour of the spots.

After development, pale yellow spots were visible in daylight, due to the fact that lumefantrine is light-yellow in colour, whereas these spots were clearly visible when viewed under UV light at 254 nm. The Ph.Int. requires at least two detection methods and although the slightly yellow spots were visible in daylight, they were not strong enough for clear identification. When the plate was exposed to iodine vapour, brown spots appeared (Figure 4.1). As staining with iodine is a fast, uncomplicated, effective and inexpensive method of detection, it was chosen as the second detection method. The longer the plate was exposed to the iodine vapour the darker the spots became. After removal of the plate from iodine exposure, the spots were clearly visible as brown spots. These spots, however, faded rather quickly and therefore a note was made in the method to assess the plates in daylight, immediately following staining.

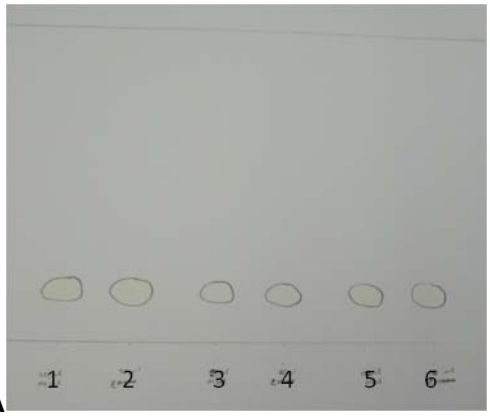
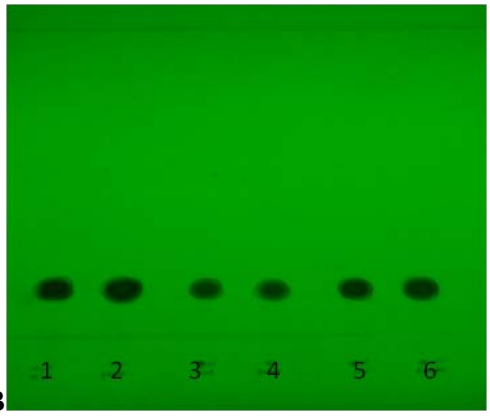
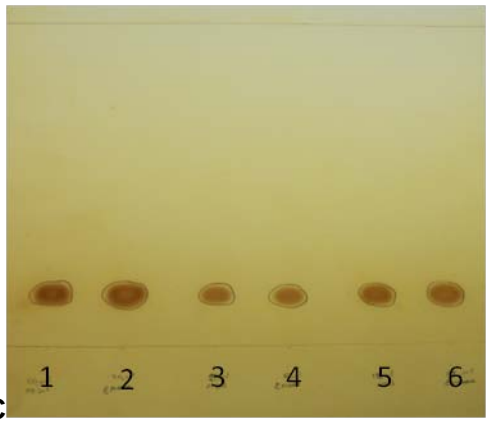
A 	Examined in daylight (pale yellow): Spot 1: MSolv, 20 µl; Spot 2: Ethyl acetate, 20 µl; Spot 3: MSolv, 5 µl; Spot 4: Ethyl acetate, 5 µl; Spot 5: MSolv, 10 µl and Spot 6: Ethyl acetate, 10 µl.
B 	Examined under UV light at 254 nm: Spot 1: MSolv, 20 µl; Spot 2: Ethyl acetate, 20 µl; Spot 3: MSolv, 5 µl; Spot 4: Ethyl acetate, 5 µl; Spot 5: MSolv, 10 µl and Spot 6: Ethyl acetate, 10 µl.
C 	Examined in daylight after exposure to iodine vapour: Spot 1: MSolv, 20 µl; Spot 2: Ethyl acetate, 20 µl; Spot 3: MSolv, 5 µl; Spot 4: Ethyl acetate, 5 µl; Spot 5: MSolv, 10 µl and Spot 6: Ethyl acetate, 10 µl.

Figure 4.1 TLC plates for lumefantrine identification in daylight, under UV light and after exposure to iodine vapour.

The acceptance criteria for the identity test in the monograph are as follows: The principal spot obtained with solution A (test solution) corresponds in position, appearance and intensity to that obtained with solution B (standard solution).

The method was validated (Appendix C) and the results obtained are summarised in Table 4.2.

Table 4.2 Summary of validation results for the TLC identification method for lumefantrine API

Parameter	Acceptance criteria	Results
Specificity	The principle spot obtained with test solution (1)[1], which is a solution of the test substance, should correspond with that of the standard solution with regards to appearance, intensity and position. The spots obtained with test solutions (2)[1] and (3)[1], which are solutions of known related substances, should not interfere with those obtained with test solution (1) and/or the standard solution. Spots should be visible under UV radiation at 254 nm. After being exposed to iodine vapour, all spots should be visible in daylight.	The principle spot obtained with test solution (1) corresponded with that of the standard solution with regards to appearance, intensity and position. The spots obtained with test solutions (2) and (3) did not interfere with the spots obtained with test solution (1), nor the standard solution. Spots were visible under UV radiation at 254 nm. After being exposed to iodine vapour, all spots were visible in daylight.
Robustness A: Stability of standard and test solutions after standing for 24 hours	The principle spots obtained with test solution (1) and the standard solution, after standing for 24 hours on the bench, should be comparable to that of freshly prepared solutions, with respect to appearance, intensity and position.	The results obtained from solutions that stood for 24 hours at ambient conditions, were comparable to the results of freshly prepared solutions.
B: Use of glass and aluminium plates	Aluminium and glass TLC plates should each produce results in which the spots of the reference standard and the test sample correspond to each other with regards to appearance, intensity and position. The Rf values of the aluminium and glass plates may differ.	Both the aluminium and glass TLC plates produced results acceptable for identification, according to the acceptance criteria.
[1] Solution (1) = test solution; solution (2) = lumefantrine related substance A; solution (3) = mixture of lumefantrine related substances B and C. Note that solutions (A) and (B), as used in the monograph, have a different meaning.		

The TLC method developed during this study for the identification of lumefantrine API, complied with the acceptance criteria set for validation and thus proved to be specific and robust. This identification test would thus allow any laboratory to identify commercial lumefantrine material in a quick and cost-effective way. The test and

sample solutions were stable for at least 24 hours and both glass and aluminium plates were suitable for the identification test.

4.2.2.2 Ultraviolet-visual (UV-VIS) spectrophotometry

Ultraviolet-visual spectrophotometry (UV-VIS) is a method widely and frequently used for the identification of APIs. The specific absorbance ($A_{1cm}^{1\%}$) of an API is a useful tool in the identification, assay and dissolution testing of APIs, or products containing them, especially in cases where the reference standards are expensive, or frequently unavailable.

For the identity test the following method was developed: Dissolve about 20 mg of the test substance, accurately weighed, in 200 ml of methanol by sonication for about 15 minutes. Allow the solution to cool to room temperature and dilute fivefold with methanol (solvent). The absorption spectrum (as described under *1.6 Spectrophotometry in the visible and ultraviolet regions* of the Ph.Int. (2008) of the diluted solution, when observed between 275 - 325 nm, would be sufficient to detect the analytical wavelength maximum at about 302 nm. The specific absorbance ($A_{1cm}^{1\%}$) was calculated as 331. For identification purposes a variation of 5% was allowed, resulting in a range of 314 – 348, as provided in the monograph.

The concentration of the test solution, when prepared as per the above method, produced an absorbance reading of about 0.5 (in the range 275 – 325 nm), which was in the ideal range for UV spectrophotometry.

The UV spectrum for lumefantrine API in methanol is illustrated in Figure 4.2, showing the spectral window for the test. Several maxima were noted between 200 - 400 nm. Normally, only one maximum is selected for the identity test (see for instance the monographs for abacavir sulfate, stavudine and nelfinavir mesilate in the Ph.Int. (2008)). The maximum at about 302 nm was selected for its clear maximum and the fact that it was at a higher wavelength.

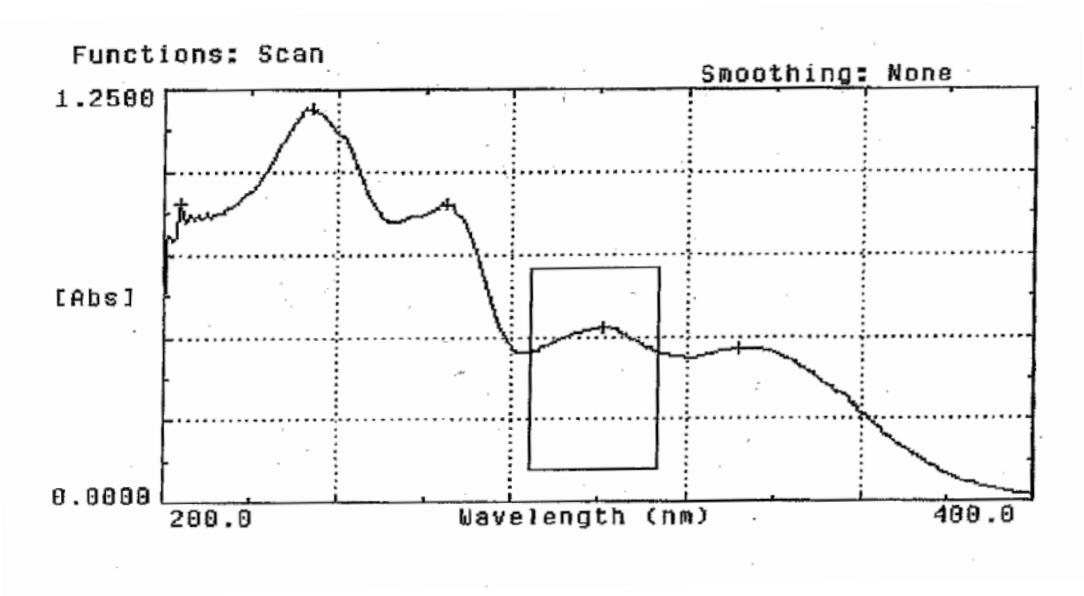


Figure 4.2 The UV spectrum of lumefantrine in the the 200 – 400 nm range.

The specific absorbance ($A_{1cm}^{1\%}$) determination of lumefantrine is discussed in Appendix D, as an alternative assay method.

4.2.2.3 Infrared (IR) spectroscopy

ICH guideline Q6A (1999:6) states that:

> *"Identification testing should optimally be able to discriminate between compounds of closely related structure which are likely to be present. Identification tests should be specific for the new drug substance, e.g. infrared spectroscopy."*

An infrared (IR) spectroscopic test is thus regarded as an impartial identification test for APIs, where the IR spectrum of the test sample is compared to that of the reference standard (or a reference spectrum provided by the Ph.Int.).

The method in the lumefantrine monograph reads (Ph.Int., 2008):

> *"Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from lumefantrine RS or with the reference spectrum of lumefantrine."*

If an API shows polymorphism, but no specific polymorphic form(s) is specified in the monograph, the method in the Ph.Int. makes provision for the possibility that the IR spectra of such polymorphic forms may differ, for example in the case of efavirenz (Ph.Int., 2008):

> *"Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from efavirenz RS or with the reference spectrum of efavirenz.* ***If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and efavirenz RS in a small amount of methanol R and evaporating to dryness.*** *The infrared absorption spectrum is concordant with the spectrum obtained from efavirenz RS."*

In developing this monograph, no provision was made for polymorphism in the description of the lumefantrine infrared identification test, since it was found (Chapter 3) that lumefantrine did not exhibit different polymorphic forms.

The IR spectrum of the lumefantrine reference standard is shown in Figure 3.14, Chapter 3.

4.2.3 Heavy metals

The test for heavy metals, as proposed by the manufacturer, was concordant with the requirements of the Ph.Int. (2008) and was thus used in this monograph.

For the limit test of heavy metals, procedure 3 (for preparation of test solution) and method A (for colour development and measurement), as described in the Ph.Int. (2008), were followed.

> *"Procedure 3: Place the quantity of the substance specified in the monograph in a suitable crucible, preferably made of silica, and carefully ignite at a low temperature until the contents are thoroughly charred. The crucible may be loosely covered with a lid during the charring. Add to the contents of the crucible 2 ml of nitric acid (~1000g/l) TS and 5 drops of suluric acid (~1760g/l) TS, and continuously heat until white fumes are evolved, and then ignite, preferably in a muffle furnace, at 500°C until all the carbon is burned off. Cool, add 2 ml of hydrochloric acid (~250 g/l) TS, and slowly evaporate in a water-bath to dryness. Moisten the residue with 1 drop of hydrochloric acid (~250 g/l) TS, add 10 ml of hot water, and digest for 2 minutes. Add, drop by drop, ammonia (~100 g/l) PbTS, until the pH of the solution is between 8 and 8.5, then add, drop by drop, acetic*

acid (~60 g/l) PbTS, to adjust the pH between 3 and 4. Filter if necessary, wash the crucible and filter with about 10 ml of water, dilute with water to 40ml, and mix.

Method A: To 40 ml of the liquid contained in the comparison tube add 10 ml of freshly prepared hydrogen sulfide TS, mix and allow to stand for 5 minutes.

In another comparison tube place a volume of solution of dilute lead PbTS, containing the lead equivalent of heavy metals limit specified in the monograph, dilute with water, adjust the pH with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3-4; dilute with water or the solvent used to 40 ml, mix, add 10 ml of freshly prepared hydrogen sulfide TS, mix and allow to stand for 5 minutes.

Compare the colours by viewing down the vertical axis of the tube in diffused light against a white background, or by another suitable method. The colour of the test solution is not darker than that of the lead standard."

Since lumefantrine is practically insoluble in water, the limit for heavy metals in lumefantrine is low (not more than 10 µg/g). For APIs that are water soluble, the limit is generally 20 µg/g (see for instance stavudine and zidovudine in the Ph.Int. (2008)).

4.2.4 Sulfated ash

For the sulfated ash determination, the procedure described in *2.3 Sulfated ash* of the Ph.Int (2008), was followed:

"Accurately weigh about 1 g of the substance into a suitable dish and moisten with sulfuric acid (~1760 g/l) TS. Heat gently to remove the excess of acid and ignite at about 800°C until all the black particles have disappeared; again moisten with sulfuric acid and re-ignite. Add a small amount of ammonium carbonate R and ignite to constant weight."

The limit for sulfated ash is not more than 1.0 mg/g, as recommended by the manufacturer, and is concordant with the normal range of limits for sulfated ash used in the Ph.Int.

4.2.5 Loss on drying (LOD)

The loss on drying (LOD) was performed on lumefantrine API (about 1 g) in a glass container, which was previously dried to constant mass. The test samples were dried to constant mass in an oven (Labotec, South Africa) at a temperature of 105ºC. Constant mass, as defined by the Ph.Int. (2008), is two consecutive weighings that do not differ by more than 0.5 mg.

The percentage loss on drying (% LOD) was calculated by means of the following equation:

$$\% \text{ LOD} = \frac{\text{Mass of sample before drying} - \text{Mass of sample after drying}}{\text{Mass of sample before drying}} \times 100$$

Table 4.3 Results obtained for lumefantrine LOD testing (performed in duplicate)

Sample	Initial mass (mg)	After 3 hours		After 4 hours	
		Mass (mg)	% LOD	Mass (mg)	% LOD
Sample 1	960.10	958.03	0.22	957.78	0.24
Sample 2	966.47	964.12	0.24	963.65	0.29
AVERAGE	-	-	0.23	-	0.27

The weight after 4 hours did not differ from that after 3 hours by more than 0.5 mg (Table 4.3). A constant mass was thus already reached after 3 hours, making it appropriate to specify 3 hours as the time for LOD testing in the monograph. The limit was set at 0.5%. This is a typical limit for non hygroscopic APIs, containing no crystal water (see for instance the monographs of efavirenz, ritonavir and stavudine in the Ph.Int. (2008)).

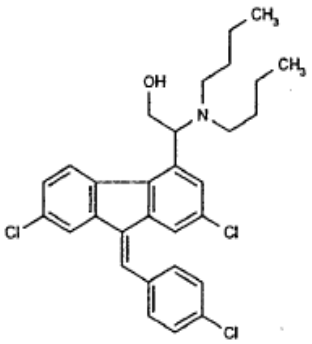
The data generated during this study (Table 4.3) supported the limit for LOD set for lumefantrine API in the Ph.Int. monograph:

"Dry for 3 hours at 105°C; it loses not more than 5.0 mg/g."

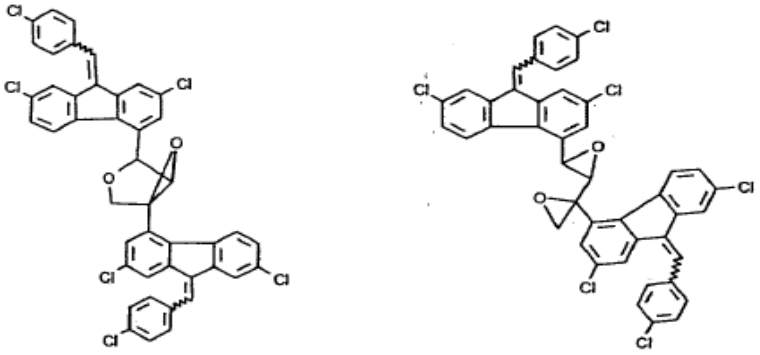
4.2.6 Related substances

Tests for related substances are used for the control of degradation products and synthesis related impurities in APIs, thus it is used to ensure the purity, quality and safety of an API, before dosage form manufacturing.

The compounds depicted in Figure 4.3 were identified by the manufacturer as synthesis related impurities of lumefantrine.



Impurity A



Impurities B and C (mixture)

Figure 4.3 Lumefantrine impurities: Lumefantrine related substance A and related substances B and C (supplied as a mixture).

The manufacturer provided (through the WHO) small amounts of related substance A and a mixture of related substances B and C (Figure 4.3).

For the monograph of lumefantrine API, two methods for related substance testing were developed:

- High-performance liquid chromatography (HPLC) method; and
- Thin layer chromatography (TLC) method.

An HPLC method is generally preferred, since it allows quantitation of the related substance, which is important for stability testing (WHO, 2009:95).

4.2.6.1 High performance liquid chromatography (HPLC)

The quantities of related substances received from the WHO for this study, were insufficient for validation of the manufacturer's method, as supplied by the WHO also. The manufacturer's validation data received for this method was evaluated and the method accepted without any changes. No results may be published for this validation method, due to the propriety rights thereof.

Stress testing was conducted on a lumefantrine test sample to determine its stability under stress conditions.

Manufacturer's method supplied by the WHO:

Column: 12.5 cm x 4.0 mm C_{18} column, 5 µm (Nucleosil-100 is suitable)

Mobile Phase A: Ion pair : water : acetonitrile : 1-propanol (200 : 500 : 250 : 50)

Mobile Phase B: Ion pair : water : acetonitrile : 1-propanol (200 : 100 : 650 : 50)

Mobile Phase C: Water : acetonitrile : 1-propanol (100 : 100 : 400)

Ion pair: Dissolve 5.65 g of sodium hexanesulfonate and 2.75 g of sodium dihydrogen phosphate in about 900 ml of water. Adjust the pH to 2.3 using phosphoric acid. Dilute to 1000 ml and filter (0.5 µm).

Solvent: Acetonitrile

Injection volume: 20 µl

Flow rate: 2.0 ml/min

Wavelength: 265 nm.

Table 4.4 Gradient table for the HPLC related substances test of lumefantrine

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comments
0-14	25	75	0	Isocratic
14-19	25 to 0	75 to 100	0	Linear gradient
19-20	0	100 to 80	0 to 20	Linear gradient
20-26	0	80	20	Isocratic
26-27	0	80 to 30	20 to 70	Linear gradient
27-50	0	30	70	Isocratic
50-51	0 to 25	30 to 75	70 to 0	Return to initial composition
51-56	25	75	0	Re-equilibration

Procedure

Use Table 4.4 to set up the gradient. Prepare the following solutions: For solution (1) use 0.3 mg/ml lumefantrine test sample. For solution (2) dilute solution (1) thousand fold (0.3 µg/ml). For solution (3) dissolve 3 mg of lumefantrine RS, containing related substances A, B and C (each about 1 mg) in 10 ml.

Relative retention times

With relation to lumefantrine, retention time = about 10 minutes. Impurity A = about 0.9; impurity B = about 4.3 and impurity C = about 4.6.

The method was conducted using a primary standard (Novartis, batch number C0189). No interfering peaks were detected in the chromatogram of the solvent (acetonitrile), as illustrated in Figure 4.4A. In the standard (Figure 4.4B) the lumefantrine active and three impurity peaks were identified. In the sample (Figure 4.4C) a lumefantrine peak was detected, but no peaks corresponded with those of the impurities. Seeing that the retention times of related substance A and lumefantrine were very close to each other (Figure 4.4B), it was decided to propose the inclusion of a peak-to-valley value as part of the system suitability requirements.

The peak-to-valley ratio is a mathematical relation, used to determine separation between two Gaussian curves. It has a larger coefficient of variation than the resolution and may thus be used in cases like this where a more sensitive separation measurement was needed. The peak-to-valley ratio is calculated, using the relation between Hp (the height above the baseline of peak A) and Hv (the height above the baseline of the lowest point of the curve separating peaks A and B) (Christophe, 1971:455).

System suitability

This test is invalid, unless the peak-to-valley ratio (Hp/Hv) is at least 2.0, where Hp = height above the baseline of the peak of impurity A and Hv = the height above the baseline of the lowest point of the curve separating this peak from that of lumefantrine.

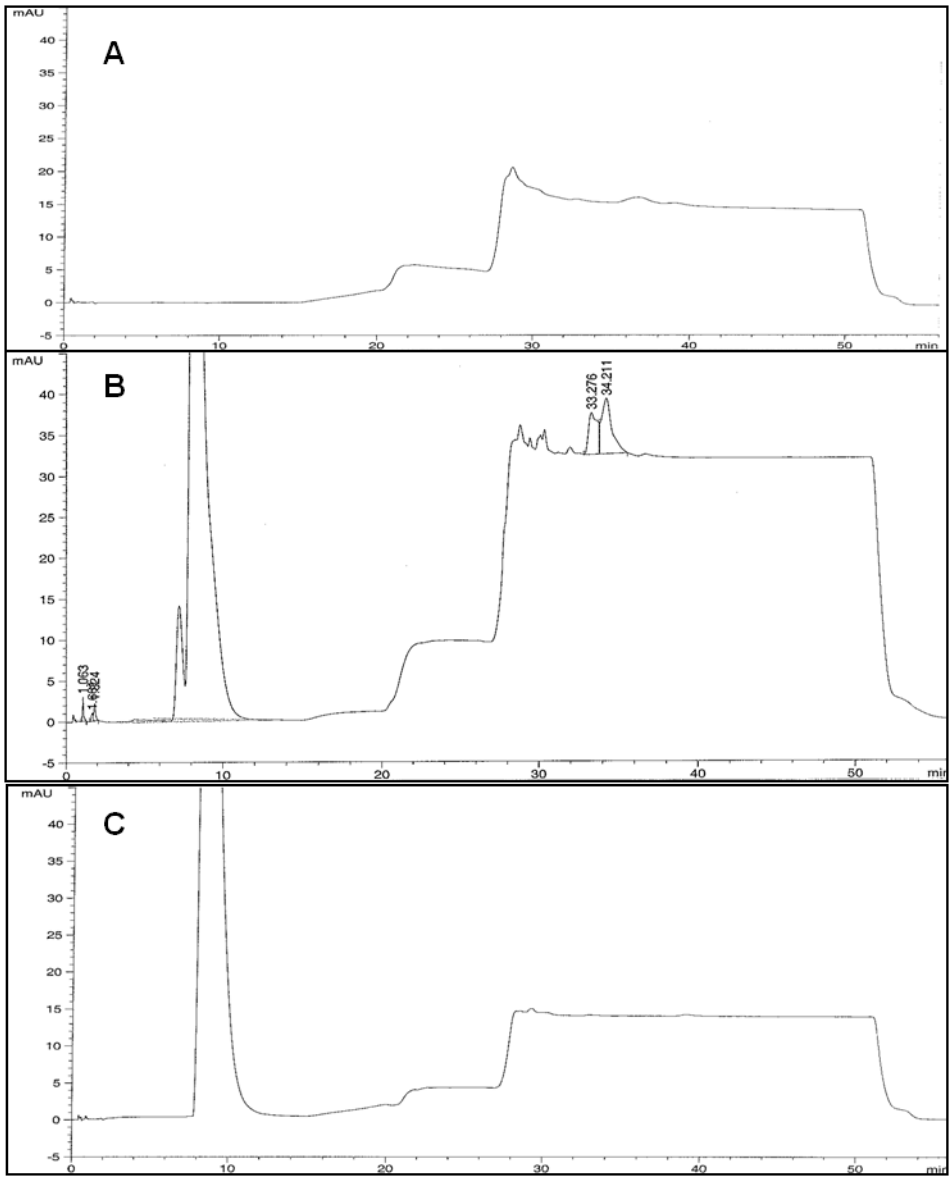


Figure 4.4 **(A)** HPLC chromatogram of the acetonitrile solvent;

(B) HPLC chromatogram of the standard solution with lumefantrine RS, related substances A (just before the main peak) and a mixture of B and C at about 33 and 34 minutes, respectively; and

(C) HPLC chromatogram of lumefantrine test sample, indicating no related substances.

The above method was proposed to the expert committee and accepted for inclusion in the lumefantrine monograph, with the addition of the peak-to-valley requirement for the system suitability.

Lumefantrine stability under stress conditions

In order to evaluate the stability of lumefantrine in solution, 6 mg of lumefantrine standard was dissolved in 10 ml of methanol. 5 ml of this solution was then further diluted with 3 ml of methanol and 2 ml of different solutions, namely 0.1 N HCl (acidic), 0.1 N NaOH (alkaline), 3% H_2O_2 (oxidative) and water, to prepare suitable media for stress testing.

These samples were stored at 20 – 25°C and analysed, using the HPLC related substance method, as discussed above. Methanol was injected in combination with each of the different solutions (in the same ratio as for sample preparation) and no interfering peaks from the solvents were detected. As seen in Table 4.5, the results for acidic, alkaline and oxidative conditions up to 36 hours showed no significant variances. The results obtained with water appeared to be anomalous, since the percentage recovery increased with time. This my have been caused by the possible evaporation of the solvent from the sample vials.

Table 4.5 Lumefantrine stability under acidic, alkaline and oxidative stress conditions

Time (hours)	Percentage recovery of lumefantrine in various media			
	0.1 N HCl	**0.1 N NaOH**	**3% H_2O_2**	**H_2O**
0	100.0%	100.0%	100.0%	100.0%
4	98.3%	98.5%	99.9%	100.5%
8	98.0%	98.9%	100.5%	101.7%
12	98.7%	100.1%	101.2%	102.9%
16	99.3%	100.7%	101.5%	103.3%
20	99.1%	100.7%	101.0%	102.8%
24	99.0%	100.0%	100.3%	102.5%
32	99.8%	100.7%	100.3%	103.4%
36	100.1%	100.7%	100.0%	103.9%

Stress studies conducted in aqueous medium indicated that lumefantrine was quite stable against hydrolytic and oxidative conditions.

4.2.6.2 Thin layer chromatography (TLC)

The method for the lumefantrine related substances of the Chinese monograph (Ch.P., 2005:487) and the method developed during this study for identification (see validation in Appendix E) (see paragraph 4.2.2.1), were evaluated as a starting point for the development of a TLC limit test for lumefantrine related substances.

Table 4.6 Chromatographic conditions of TLC methods used in the development of a limit test for lumefantrine related substances

	Chinese monograph	**Developed method for inclusion in monograph**
Coating of TLC plate	Silica gel 60, F 254	Silica gel 60, F 254
Solvent	Chloroform	Ethyl acetate
Mobile phase	n-Hexane : acetone : diethylamine (40 : 7 : 3) (v/v)	Petroleum ether (BP 40 - 60ºC) : ethyl acetate : glacial acetic acid (40 : 6 : 10) (v/v)
Test solutions (mg/ml of lumefantrine)	Solution (1) 10 mg/ml Solution (2) 0.05 mg/ml	Solution (1) 10 mg/ml Solution (2) 0.1 mg/ml Solution (3) 0.03 mg/ml Solution (4) 0.01 mg/ml
Application volume	10 µl	10 µl
Detection A	UV at 254 nm	UV at 254 nm
Detection B	Expose to iodine vapour until spots appear and assess immediately in daylight.	N/A
Evaluation	Any spot in the chromatogram, other than the principal spot obtained with solution (1) is not more intense than the principal spot obtained with solution (2) (0.5%) and not more than 4 spots are observed.	No spot, other than the principal spot obtained by solution (1) should be more intense than that in the chromatogram obtained with solution (3) (0.3%) and not more than two such spots may be more intense than that obtained by solution (4) (0.1%).

Method development

The use of chloroform as solvent and diethylamine in the mobile phase in the Ch.P. (2005:487) method are discouraged by the WHO (2005), due to their toxicity. The possibility of combining the newly developed method for identification of lumefantrine with the related substances method, in order to obtain one method for both the identification and related substance tests, was investigated.

The mobile phase combination of the manufacturer (Table 4.1) for the identification of lumefantrine, did not allow adequate separation of the related substances. The mobile phase was thus adjusted to obtain better separation of the related substances, as illustrated in Table 4.6. In Figure 4.5, some of the combinations are illustrated with TLC plates A, B and C, while plate D illustrates the final mobile phase, as included in the monograph.

The related substances were dissolved in chloroform, as well as in ethyl acetate, and both were found to be effective solvents. Ethyl acetate was selected as the most suitable solvent during the method for validation (as discussed in section 4.2.2.1). Spots were largely invisible in daylight, but clear under UV at 254 nm. See validation results in Table 4.7.

Table 4.7 Summary of validation results for lumefantrine related substances TLC method

Parameter	Acceptance criteria	Results
Specificity	Spots produced by solutions (A)[1], (B)[1] and (C)[1] should be clearly visible under UV light 254 at nm. Spots due to solutions (A), (B) and (C) must have different Rf values to allow clear separation, whilst solution (C) should produce 2 spots.	The spots appeared as clearly visible, dark, round to oval shaped spots under UV light at 254 nm. Spots due to reference solutions (A), (B) and (C) had different Rf values and illustrated good separation.
Detection limit (DL)	The spot obtained for the 0.1% solution should be clearly visible.	The spot for 0.1% was clearly visible.
Robustness A: Stability of standard and test solutions after standing for 24 hours	The spots observed on the plates obtained from the aged solutions, should be similar to those obtained from freshly prepared solutions.	The results produced by the aged sample corresponded with the freshly prepared sample with regards to appearance, intensity and position (Rf) and the solutions proved to be stable for at least 24 hours.
B: Use of glass and aluminium plates by two analysts	The aluminium and glass TLC plates should produce results corresponding to each other with regards to appearance, intensity and position (RRf).	Both the aluminium and glass TLC plates produced results acceptable for identification, according to the acceptance criteria (Tables 3 and 4).
[1] Solution (A) = test solution; solution (B) = related substance A and solution (C) = related substances B and C.		

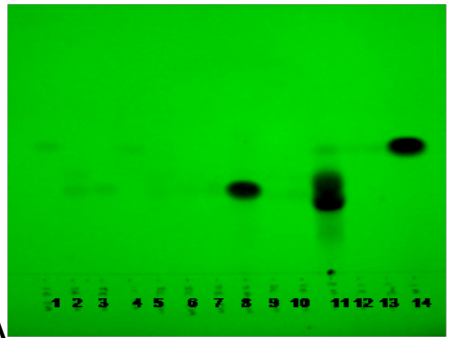
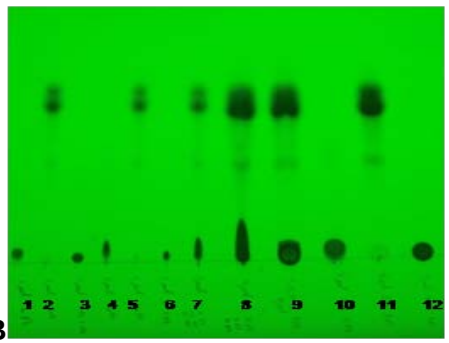
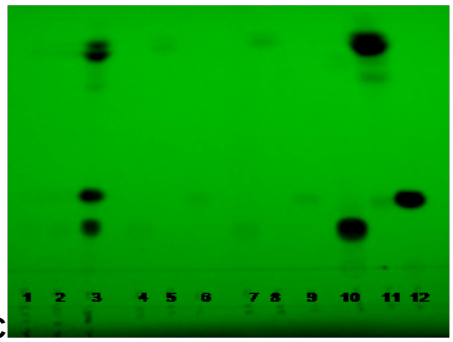
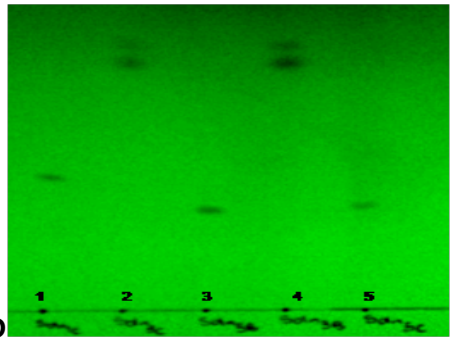
A 	Mobile phase: n-hexane : acetone : diethylamine (40 : 7 : 12) (% v/v) Solvent: ethyl acetate Spot volumes: 1, 3 and 5 µl
B 	Mobile phase: petroleum ether (BP 40-60ºC) : ethyl acetate : glacial acetic acid (40 : 5 : 0.5) (% v/v) Solvents: chloroform and ethyl acetate Spot volumes: 1, 3 and 5 µl
C 	Mobile phase: petroleum ether (BP 40-60ºC) : ethyl acetate : glacial acetic acid (40 : 7 : 5) (% v/v) Solvents: chloroform and ethyl acetate Spot volumes: 1, 3, 5 and 10 µl
D 	Mobile phase: petroleum ether (BP 40-60ºC) : ethyl acetate : glacial acetic acid (40 : 6 : 10) (% v/v) Solvent: ethyl acetate Spot volumes: 10 µl.

Figure 4.5 TLC plates for the development of a limit test for lumefantrine related substances.

As in the case of the Chinese Pharmacopoeia monograph (Ch.P., 2005:487), the related substances were tested, using a dilution of the API test solution, with strengths representing those of the limits for the related substances.

This method was developed in order to allow a laboratory to perform the limit test of lumefantrine related substances, without the need to buy the expensive and possibly not readily available related substances. This TLC method was included in the lumefantrine monograph as an alternative to the HPLC method for lumefantrine related substances, for which the related substance standards were needed.

4.2.7 Assay

A non aqueous titration method (section 4.2.7.1) and a UV spectrophotometry method (section 4.2.7.2), using specific absorbance ($A_{1cm}^{1\%}$), were developed for the assay of lumefantrine API. The non aqueous titration method was included into the monograph.

4.2.7.1 Titrimetry

Titration is a relatively inexpensive, accurate and easy technique for assay of APIs and is therefore one of the techniques regularly used by the major pharmacopoeias. The validation of the non aqueous titration method to determine the potency of lumefantrine API is discussed in Appendix F (summary of results, Table 4.8), and was also the proposed assay method in the monograph.

When compared to spectrophotometric analysis, HPLC and bioassay, non aqueous titration methods are clearly the least costly, less timeous, and requiring no expensive equipment, nor specialised technicians, making these methods part of most laboratories' routine analyses (Marona & Schapoval, 2001:229).

Many compounds that are insoluble in water attain enhanced acidic or basic properties when dissolved in organic solvents, resulting in the possible determination of these compounds by non aqueous titration. The types of compounds that may be titrated as bases include amines, with the preferred volumetric solution being perchloric acid in glacial acetic acid (USP, 2010). As lumefantrine is an amine base, non aqueous titration with perchloric acid was a feasible option during this study.

For determination of the potency of lumefantrine test samples titrimetrically, a Metrohm 785 DMP Titrino, with a Metrohm Solvatrode (LiCl in ethanol) electrode was used.

The method for titration accepted in the monograph (Ph.Int. 2008) was as follows:

> *"Dissolve about 0.45 g, accurately weighed, in 50 ml of glacial acetic acid R1 by stirring for about 15 minutes, and titrate with perchloric acid (0.1 mol/l) VS, determine the end-point potentiometrically as described under 2.6. Non aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/ml) VS is equivalent to 52.98 mg of $C_{30}H_{32}Cl_3NO$."*

The method, as described in section *2.6 Non aqueous titration, method A* in the Ph.Int. (2008) is as follows:

"Method A (for bases and their salts)

Prepare a solution as specified in the monograph or dissolve the substance being examined in a suitable volume of glacial acetic acid R1, previously neutralized to crystal violet/acetic acid TS, warming and cooling if necessary. Alternatively the titration blank for the solvent and indicator may be established in a separate determination. When the substance is a salt of a hydrohalic acid, add 10 ml of mercuric acetate/acetic acid TS. When the end-point is determined visually by colour change, add 2-3 drops of crystal violet/acetic acid TS, and titrate with perchloric acid of the specified concentration (mol/l) to the appropriate colour change of the indicator. When a different indicator is specified in the monograph, this indicator should also be used for the neutralization of the glacial acetic acid R1, and mercuric acetate/acetic acid TS, and the standardization of the titrant.

When the equivalence point is determined potentiometrically, the indicator is omitted and neutralization of the solution and standardization of the titrant are also carried out potentiometrically. A glass electrode and a saturated calomel cell (containing potassium chloride (350 g/l) TS) as reference electrode, are used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be a minimum of transfer of liquid from one side to the other. Serious instability may result unless the connections between the potentiometer and the electrode system are in accordance with the manufacturer's instructions.

When the temperature (t_2) at which the titration is carried out differs from the temperature (t_1) at which the titrant was standardized, multiply the volume of the titrant required by [1 + 0.001(t_1 - t_2)] and calculate the result of the assay from the corrected volume."

Table 4.8 Summary of validation results for the non aqueous titration assay method

Parameter	Acceptance criteria	Results
Linearity & Range	The method should be linear over a range of 80 – 120%, with the correlation coefficient (R^2) ≥ 0.99.	The method was linear over a range of 80 – 120% and R^2 = 0.9993.
Accuracy	The mean recovery must be ± 1% of the theoretical 100% in all three series.	The mean recovery was between 99.50 - 101.50%, with an average of 100.5% in 9 samples.
Precision		
A: Repeatability	The %RSD must be ≤ 1.0%.	The %RSD = 0.5%.
B: Intermediate precision	The %RSD must be ≤ 2.0%.	The %RSD = 0.4%.

As the titration method is not regarded as highly specific, it should be complemented by either a TLC or an HPLC related substances test. The limits, as set in the monograph, were 98.5 – 101.0%. The rationale for these limits is discussed under 4.2.1.

4.2.7.2 UV-VIS spectrophotometry

An alternative assay method for lumefantrine was developed, using UV spectrophotometry with the specific absorbance ($A_{1cm}^{1\%}$) value. This method was developed in conjunction with the identification test and could be used in cases where a reference standard is not readily available. The validation of this method is discussed in Appendix D and the validation results are summarised in Table 4.9.

Table 4.9 Summary of validation results for UV-VIS spectrophotometry method

Parameter	Acceptance criteria	Results
Specificity	The spectrum of test solution must conform to that of the standard solution.	The spectrum of the test solution conformed to that of the standard solution and the solvent was used as blank.
Linearity & Range	The method should be linear over a range of 60 – 120% and the correlation coefficient $R^2 \geq 0.99$.	The method was linear over a range of 60 – 120% and $R^2 = 0.9999$.
Accuracy	The difference between the percentage mean recovery and the theoretical (100%) must be ± 2.0% for all six solutions (percentage recovery should be 98.0 – 102.0%).	The average of the six solutions was 99.72% and the percentage recovery for all six solutions was between 98.8 – 100.8%.
Precision		
A: Repeatability	The % RSD must be ≤ 1.0%.	% RSD = 0.7%.
B: Reproducibility	The percentage difference between the laboratories should be ≤ 2.0%.	The percentage difference between laboratories = 0.2%.
Robustness	The samples should show a maximum deviation of ≤ 1.0% from the initial value after 48 hours.	The samples showed a deviation of 0.3% from the initial value after 48 hours.
Determination of $A_{1cm}^{1\%}$	Use the accuracy data to calculate the $A_{1cm}^{1\%}$ value.	$A_{1cm}^{1\%}$ = 331.4

The method proved to be specific and linear within the range of 60 – 120%. Accuracy was proven and precision testing proved the method to be repeatable and reproducible (between laboratories). The samples proved to be stable for at least 48 hours at ambient conditions. The assay values obtained for the same sample tested, using the titration and UV methods, produced similar results, i.e. 100.2% and 100.1%, respectively (Table 6 in Appendix F).

4.3 Conclusion

This chapter outlined the general information contained in the proposed lumefantrine API monograph, and described the development of the methods, as defined under “Requirements” of the monograph.

For the identification test, either infrared could be used alone, or a combination of UV spectrophotometry and TLC could be performed.

The proposed tests for heavy metals, sulfated ash and loss on drying were standard pharmacopoeial tests.

Two tests were proposed for related substances, namely an HPLC method and a newly developed TLC test.

For the assay of lumefantrine, titration with perchloric acid was decided upon. As alternative, and supplementing the identification test, an UV-VIS test, using specific absorbance ($A_{1cm}^{1\%}$), was developed.

The main focus during the development of this monograph was to produce effective methods, typically used in pharmacopoeial monographs, for testing of lumefantrine API, in order to ensure the availability of good quality raw materials.

The validation procedures and outcomes of the methods being developed are outlined in appendices. The TLC identification method is attached in Appendix C, that of the UV-VIS identification method and specific absorbance ($A_{1cm}^{1\%}$) in Appendix D, the TLC for related substances in Appendix E and the titration assay in Appendix F.

The HPLC related substances method was performed and included results of the stability (stress) testing conducted. This also proved lumefantrine to be stable under stress conditions for at least 36 hours.

The final monograph for possible inclusion in the Ph.Int. is described in Appendix B.

CHAPTER 5

Monograph development for the artemether and lumefantrine FDC dosage forms

5.1 Introduction

The availability of monographs for artemether/lumefantrine FDCs, as discussed in Chapters 1 and 2, plays an important role in the fight against malaria.

The motivation behind combining antimalarials with different modes of action is twofold: (1) the combination often is more effective, and (2) in the incident of a parasite being resistant to one of the APIs, the parasite would be killed by the other. This mutual protection is considered to prevent or delay resistance. To ensure efficiency, the APIs in a FDC must be independently effective, as is the case with artemether and lumefantrine (WHO, 2006:22).

During the development processes, draft specifications were sent to the expert panel and specialists of the WHO for peer reviewing. All feedback was considered and included in the specifications, where appropriate.

This chapter provides information regarding existing methods being used, about newly developed methods and the validation thereof, as well as on the requirements set in the monographs for artemether/lumefantrine tablets and powder for suspension.

These proposed FDC monographs, as adopted for inclusion in the Ph.Int., second supplement, by the WHO Expert Committee on Specifications for Pharmaceutical Preparations (held in Geneva during October 2007), are included in Appendix B. These adopted monographs were published in July of 2008 on the official WHO website:

- Monograph for artemether and lumefantrine tablets (section 5.2):

  http://www.who.int/medicines/publications/pharmacopoeia/Lum-art-tabs-monoFINALQAS07192July2008.pdf.

- Monograph for artemether and lumefantrine oral suspension[1,2] (section 5.3):

  http://www.who.int/medicines/publications/pharmacopoeia/QAS07-217FINALArtem-Lumefan-oralsol.pdf.

5.2 Specifications developed for the artemether/lumefantrine tablets monograph

The components of monographs for tablets, according to the Ph.Int. (2008), as listed in Chapter 2 (Table 2.1), were used as a guideline for selecting suitable methods for the development of a monograph for artemether/lumefantrine tablets.

The requirements for the monograph included the following (refer Table 2.1):

- Definition;
- Identity tests (TLC and HPLC);
- Artemether related substances (TLC); and
- Assay (HPLC).

5.2.1 Definition

The monograph for artemether/lumefantrine tablets should comply with the monograph for "Tablets" in the Ph.Int. (2008).

This definition, as included in the monograph, is in accordance with the requirements generally set by die Ph.Int. for tablets:

> *"Artemether and Lumefantrine tablets contain Artemether and Lumefantrine. They contain not less than 90.0% and not more than 110.0% of the amounts of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) stated on the label."*

[1] "Artemether and lumefantrine oral suspension" is a suspension of artemether and lumefantrine in a suitable vehicle; it may be flavoured. It is prepared by suspending the powder in the specified volume of the liquid stated on the label just before issue for use.
[2] In this thesis the dosage form is called "artemether/lumefantrine powder for suspension".

5.2.2 Identification

As indicated in section 4.2.2, in the absence of a highly specific method, such as infrared (IR) spectrophotometry, further identification tests may collectively be regarded as sufficient (Ph.Int., 2008).

For the monograph of artemether/lumefantrine tablets, two methods for identification were developed:

- A thin layer chromatography (TLC) method and; and
- A high performance liquid chromatography (HPLC) method.

5.2.2.1 Thin layer chromatography (TLC)

In planar chromatographic methods, such as TLC, chromatography is effected by allowing the mobile phase to flow over and through a layer of the adsorbent. Planar methods are simple and effective and require inexpensive equipment, although some complex accessories are available. These methods are of enormous value during screening and identification tests, but are less suitable for precise, quantitative determinations (Ph.Int., 2008).

An existing TLC method for artemether/lumefantrine tablets from a manufacturer, as supplied by the WHO, was evaluated. Some adjustments were made prior to successful validation of the method (Appendix G) and was included in the monograph (Appendix B).

General procedure for TLC testing

The TLC test applied for identification purposes was performed using commercially available, pre-coated, TLC plates as stationary phase. The general procedure, as described under section *1.14.1 Thin-layer chromatography* of the Ph.Int. (2008), was followed (see section 4.2.2.1).

As indicated in section 4.2.2, it is quite standard for Ph.Int. monographs to have two methods of detection of TLC spots. The method developed for the artemether/lumefantrine tablets monograph accordingly also had two procedures, which were similar in chromatography, but which differed with regards to the detection of the spots, namely through inspection under UV light, or in daylight.

In Table 5.1, the original method from the manufacturer and that of the adjusted method A.1, as included in the final monograph, are compared.

As indicated in Table 5.1, one standard solution was prepared for the new Ph.Int. monograph test, in contrast with the two standard solutions that had been used by the manufacturer. Separate standard solutions would normally be preferred, since this would allow for the individual identification of each API. The same outcome was in fact achieved by method A.1, since each detection procedure was able to identify a particular API (see Table 5.1).

Method A.2 of the Ph.Int. monograph utilised the same chromatographic procedure, but the detection of the spots differed:

> *"Spray with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C, allow it to cool and expose to iodine vapours for 20 minutes. Examine the chromatogram immediately in daylight.*
>
> *The principal spots obtained with solution A* (test solution in Table 5.1) *correspond in position, appearance, and intensity to those obtained with solution B* (standard solution (1) in Table 5.1)*."*

In contrast with method A.1, method A.2 did not allow for the individual identification of the APIs, since they were simultaneously detected. Method A.1 was, however, the method of choice, as indicated in the monograph text (Appendix B): "Carry out test A.1, or where UV detection is not available, test A.2."

Table 5.1 Comparison of two TLC methods for the identification of artemether/lumefantrine tablets

	Existing method from manufacturer for artemether/lumefantrine tablets (supplied by WHO)	**Method A.1 for inclusion in artemether/lumefantrine tablets monograph**
Layer	Silica gel 60, F 254	Silica gel 60, F 254
Solvent	Water : ethyl acetate : methanol : chloroform (2 : 2 : 10 : 11) (v/v)	Acetone
Mobile phase	Light petroleum ether (boiling point: 40 - 60ºC) : ethyl acetate : glacial acetic acid (40 : 10 : 5) (v/v)	Petroleum ether (boiling point: 40 - 60ºC) : ethyl acetate : glacial acetic acid (40 : 10 : 5) (v/v)
Test solution[1]	Artemether = 0.8 mg/ml; Lumefantrine = 4.8 mg/ml	Artemether = 1.0 mg/ml; Lumefantrine = 6.0 mg/ml
Standard solution (1)[1]	Artemether = 0.8 mg/ml	Artemether = 1.0 mg/ml; Lumefantrine = 6.0 mg/ml
Standard solution (2)	Lumefantrine = 4.8 mg/ml	–
Application volume	20 µl	10 µl
Detection A **Evaluation**	UV, 254 nm Assess the agreement of the spots for lumefantrine in the test solution and standard solution (1) with respect to Rf value, colour, and approximate size. Lumefantrine appears as a dark spot on a light fluorescent background.	UV, 254 nm The principal spot obtained with the test solution corresponds in position, appearance, and intensity to that obtained with standard solution (1) (identifying lumefantrine).
Detection B **Evaluation**	Spray the chromatogram with sulfuric acid in methanol 20% (v/v), heat for 10 minutes in a drying oven at 140°C and assess in daylight. Assess the agreement of the spots for artemether in the test solution and standard solution (1) with respect to Rf value, colour, and approximate size. Artemether appears as a greyish-purple spot on a white background.	Spray the chromatogram with sulfuric acid in methanol 10% (v/v), heat for 10 minutes at 140°C in a drying oven, and assess in daylight. The principal spot obtained with the test solution corresponds in position, appearance, and intensity to that obtained with standard solution (1) (identifying artemether: a faint spot, but due to lumefantrine may also be visible).
Detection C **Evaluation**	After detection B, assess under UV light at 366 nm. Artemether appears as a light yellow fluorescent spot on a blue background in detection C, Rf value: about 0.6, while lumefantrine appears as a dark spot on a blue fluorescent background in detection C, Rf value: about 0.15.	–

[1] In the monograph the test solution is defined as solution A and standard solution (1) is defined as solution B.

Development of a TLC method for the identification of the APIs in artemether/lumefantrine tablets

The manufacturer's method for the identification of artemether and lumefantrine in artemether/lumefantrine tablets was adjusted substantially, in order to obtain an uncomplicated, practical method for inclusion in the Ph.Int. monograph (Table 5.1 for method A.1).

Firstly, a change in the solvent (Msolv) [water : ethyl acetate : methanol : chloroform (2 : 2 : 10 : 11) (v/v)] of the manufacturer's method was investigated. Acetone was considered as a single solvent, mainly because it would exclude the possibility of an error in solvent preparation and as it would eliminate the use of chloroform, which is discouraged by the WHO in QC testing, due to its toxicity (Ph.Int., 2005). Acetone was found to be an effective solvent. As can be seen in Figure 5.1, the spots obtained when using acetone, were similar in appearance, intensity and position (Rf) to the spots obtained, when using the manufacturer's method.

Secondly, the volumes of the standard and test solutions applied were studied, by using slightly stronger solutions than the manufacturer. In Figure 5.1 the effect of different spot volumes is illustrated. 10 µl was chosen as a suitable volume for the identification test of artemether and lumefantrine, based on the shape, intensity and colour of the spots.

The methods of detection were further investigated in order to match the Ph.Int. custom of having two methods of detection of TLC spots.

Detection by means of UV at 254 nm was firstly investigated (method A.1). Lumefantrine produced a strong spot when viewed under UV light. However, artemether lacks a chromophore (or any double bond) in its structure, which caused it to be invisible when viewing the TLC plate under UV light. Accordingly, direct investigation under UV light at 254 nm would selectively identify lumefantrine. In order to obtain a spot for artemether, the manufacturer's detection method B was pursued. It was found that after treatment of the plates with sulfuric acid in methanol, the artemether spots were clearly visible when viewed in daylight (Figure 5.1). The spots for lumefantrine in daylight was only slightly visible. The investigation of the plate in daylight, after treatment with sulfuric acid in methanol, thus proved to be selective with respect to the identification of artemether. Method A.1 could thus be regarded as very specific.

Subsequently, a second detection method, using only inspection in daylight, was investigated (method A.2). Lumefantrine showed faint yellow spots on the plate in the absence of any further treatment, when viewed in daylight. As lumefantrine is slightly yellow in colour, the spots were not considered sufficiently intense for distinct identification. From experience during the development of method A.1, it was decided to expose the plate, after treatment with sulfuric acid in methanol, to iodine vapour, considering the convenience and low cost of iodine staining. The outcome was positive (Figure 5.1) and the longer the plate was exposed to iodine vapour the darker the spots became. After removing the plate from the iodine chamber, the spots for both APIs were clearly visible as brown spots. These spots, however, faded rather quickly and therefore a note was made in the method to assess the plates in daylight, immediately following staining.

The spray reagent, sulfuric acid in methanol 20% (v/v), which had not been defined in the Ph.Int., was investigated for possible substitution by sulfuric acid in methanol 10% (v/v) [*Sulfuric acid/methanol TS* in the Ph.Int.]. *Sulfuric acid/methanol TS* was indeed found to be effective as a spray reagent.

The methods were validated (see Appendix G) and the results obtained are summarised in Table 5.2.

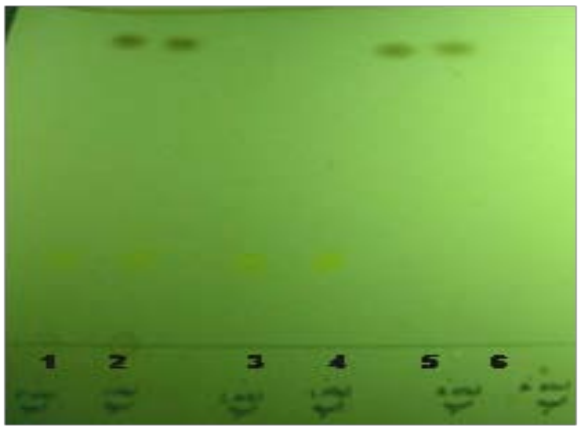
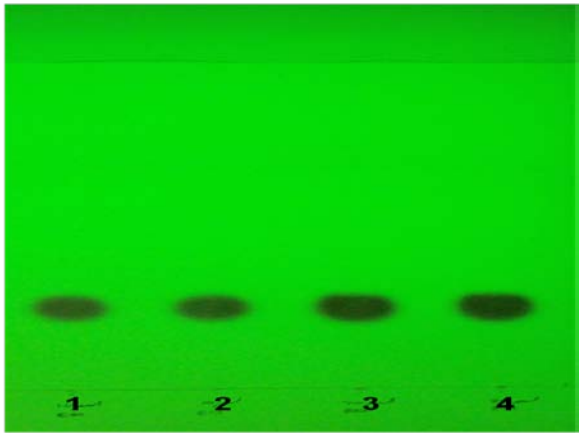
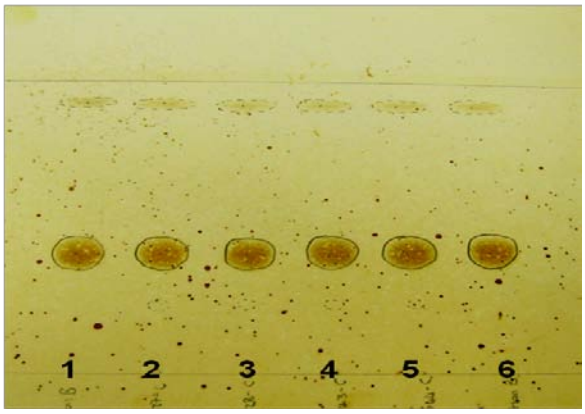
A  	Method A.1 in daylight after treatment with sulfuric acid in methanol: Spot 1: Test solution (solution A); Spot 2: Standard solution (1) (solution B); Spot 3: Standard solution (1) (lumefantrine) in acetone; Spot 4: Standard solution (1) (lumefantrine) in Msolv; Spot 5: Standard solution (2) (artemether) in acetone; Spot 6: Standard solution (2) (artemether) in Msolv.
B  	Method A.1 in UV light at 254 nm: Spot 1: Solution B (10 µl); Spot 2: Solution A (10 µl); Spot 3: Solution B (20 µl); Spot 4: Solution A (20 µl).
D  	Method A.2 in daylight after iodine staining: Spot 1: Solution B (20 µl); Spot 2: Solution A (20 µl); Spot 3: Solution A (20 µl); Spot 4: Solution A (20 µl); Spot 5: Solution A (20 µl); Spot 6: Solution B (20 µl).

Figure 5.1 TLC plates for the method development of the identification of artemether and lumefantrine.

Table 5.2 Summary of validation results for the TLC identification method

Parameter	Acceptance criteria	Results
Specificity	The principle spot obtained in the chromatogram with the test solution detected under UV light at 254 nm (before spraying) should correspond to the principle spot of the standard solution representing lumefantrine, in position (Rf), appearance and intensity.	The principle spot of the test solution detected under UV light at 254 nm (before spraying) corresponded to the principle spot of the standard solution representing lumefantrine, in position (Rf), appearance and intensity.
	The principle spot obtained with the test solution after spraying and heating corresponds to the principle spot obtained with the standard solution representing artemether, in position (Rf), appearance and intensity.	The principle spot obtained with the test solution after spraying and heating corresponded to the principle spot obtained with the standard solution representing artemether, in position (Rf), appearance and intensity.
	The two principle spots detected from the test solution after spraying and exposure to iodine vapour should correspond to those of the standard solution in position (Rf), appearance and intensity.	The two principle spots detected from the test solution after spraying and exposure to iodine vapour corresponded with those of the standard solution in position (Rf), appearance and intensity.
	Any spots detected from the placebo solution should not interfere with that detected with the standard solution.	No spots were detected with the placebo solution.
Robustness	The principle spots obtained with the freshly prepared and aged solutions should correspond with regards to appearance, intensity and position (Rf) using all three detection methods.	The results obtained after solutions stood for 48 hours corresponded with those of the freshly prepared solutions for all three detection methods.
	The Rf values of the aluminium and glass plates may differ.	The Rf values of the aluminium and glass plates differed significantly.

The method proved to be specific and robust with respect to both artemether and lumefantrine APIs, when using aluminium or glass TLC plates. The excipients did not interfere with the test results, whilst the standard and test sample solutions were stable for at least 48 hours at ambient conditions.

A TLC method for the identification of artemether and lumefantrine in artemether/lumefantrine tablets had thus been successfully developed and validated. According to the general requirements of the Ph.Int., two methods were developed to expose the lumefantrine TLC spots obtained.

5.2.2.2 High -performance liquid chromatography (HPLC)

The identification method with HPLC formed part of the assay method, as discussed in section 5.2.4.1.

The acceptance criterion for this identity test in the monograph was as follows:

> *"The retention times of the two principal peaks in the chromatogram obtained with solution (1) [sample solution] correspond to those in the chromatogram obtained with solution (2) [standard solution]."*

5.2.3 Related substances

In section 4.2.6.1, a lumefantrine sample was exposed to stress conditions and found to be stable in water and under acidic, alkaline and oxidative conditions for up to 36 hours. Since lumefantrine was found more stable than artemether (section 5.2.4), only the testing for the related substances of artemether was needed for inclusion in the monograph. The next section describes the TLC method, as developed for the artemether related substances limit test.

5.2.3.1 Thin layer chromatography (TLC) limit test for artemether related substances

An existing method of a manufacturer for the identification of the APIs in artemether/lumefantrine tablets, as supplied by the WHO, was evaluated and some adjustments made, prior to validation thereof (Appendix H) for inclusion in the proposed monograph, as is discussed below (Ph.Int., 2008).

> *"Artemether-related substances. Protect samples from light, also during chromatography.*
>
> *Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 40 volumes of light petroleum R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R as the mobile phase.*
>
> *Prepare the following solutions in the solvent consisting of 1 volume of water R and 1 volume of acetonitrile R. For solution (1), weigh and powder 20 tablets. To a quantity of the powder containing 100 mg of Artemether add 20 ml of the solvent, sonicate for 15 minutes and centrifuge. Filter a portion of the supernatant through a 0.45-µm filter, discarding the first few ml of the filtrate.*

For solution (2) dissolve 5 mg of each of artemether RS, artenimol RS and α-artemether RS in 50 ml of the solvent. For solution (3) dilute 2.0 ml of solution (2) to 20 ml with the solvent. For solution (4) dilute 3.0 ml of solution (2) to 20 ml with the solvent. For solution (5) dilute 5.0 ml of solution (2) to 20 ml with the solvent. For solution (6) dilute 1.0 ml of solution (2) to 2 ml with the solvent. For solution (7) dilute 3.0 ml of solution (2) to 4 ml with the solvent. Apply separately to the plate 20 µl of each of solutions (1), (3), (4), (5), (6) and (7). After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in vanillin/sulfuric acid TS2. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight.

Artemether and related substances have the following Rf values: impurity A about 0.25; impurity B (artenimol) about 0.3; impurity C about 0.35; impurity D (α-artemether) about 0.4; artemether about 0.55. The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

In the chromatogram obtained with solution (1):

– any spot corresponding to impurity A is not more intense than the principal spot in the chromatogram obtained with solution (7) (1.5%),

– any spot corresponding to impurity B is not more intense than the spot due to artenimol in the chromatogram obtained with solution (6) (1.0%),

– any spot corresponding to impurity C is not more intense than the principal spot in the chromatogram obtained with solution (5) (0.5%),

– any spot corresponding to impurity D is not more intense than the spot due to α-artemether in the chromatogram obtained with solution (4) (0.3%),

– any other spot is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot remaining at the point of application."

Apart from minor changes made to the preparation of the test solution, the following adjustments were made to the manufacturer's method.

Firstly, the spray reagent was changed from sulfuric acid in methanol (20% v/v) to *vanillin/sulfuric acid TS2*, as per the Ph.Int. (2008). The benefit of this proposed change was confirmed by an independent WHO collaborating laboratory, as being efficient. The spots for the artemether related substances were weak and if the spray

technique was applied, it had the tendency of causing small spots on the plate, which had the potential of influencing the visibility of related substances at low concentrations. The spray reagent was thus used to dip the plates in, instead of spraying them (Figure 5.2). The dip procedure proved to be effective and was selected for the final method.

With the high concentration of lumefantrine in the tablets, it was expected that lumefantrine may interfere with the artemether related substances TLC method. Since lumefantrine is practically insoluble in acetonitrile : water (1 : 1), it was chosen as solvent. In contrast, it was found that after dissolving lumefantrine in ethyl acetate and applying it to the plate, a big yellow spot was visible (Figure 5.2). In Figure 5.2, a sample of 120 mg lumefantrine and an analytical placebo[1], equivalent to the mass of one tablet, were treated according to the sample preparation procedure in the monograph and applied to the plate. There was no spot for lumefantrine, nor for any of the excipients in the placebo visible. This method thus proved to be specific with regards to the artemether related substances.

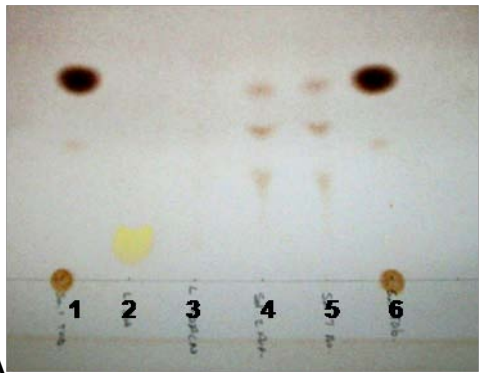
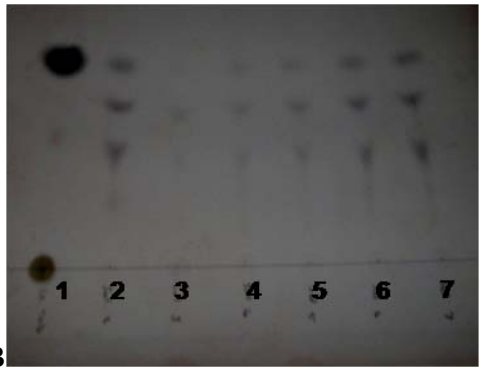
A 	Plate sprayed with *vanillin/sulfuric acid (TS2)* in daylight: Spot 1 and 6: Solution (1) test sample in solvent (water : acetonitrile); Spot 2: Lumefantrine in ethyl acetate; Spot 3: Lumefantrine in water : acetonitrile; Spot 4 and 5: Dilutions of artemether and related substances (solution (2)).
B 	Plate dipped with *vanillin/sulfuric acid (TS2)* in daylight: Spot 1: Test sample in water : acetonitrile; Spots 2 to 7: Related substances; (solutions 2[2] to 7, dilutions prepared according to method included in monograph).

Figure 5.2 TLC plates for the artemether related substances in artemether/lumefantrine tablets.

[1] The qualitative analytical placebo was prepared based on the information provided by the product information of Riamet®, see Appendix G.
[2] Solution 2 was used for the specificity testing in the validation process.

Table 5.3 Summary of validation results of the TLC method for artemether related substances

Parameter	Acceptance criteria	Results
Specificity	Solution (2)[1] should produce three spots with different Rf values to allow clear separation. Spots produced by solutions (1) and (2) should be clearly visible in daylight, solutions (A)[2] and (B)[3] should preferably show no spot. Spots due to solutions (A) and (B) should show no interference with any spot produced by solution (2).	Solution (2) produced three spots with different Rf values. Spots produced by solutions (1) and (2) were clearly visible in daylight. Solutions (A) and (B) showed no spots. Solutions (A) and (B) showed no spots, thus no interference with any spot produced by solutions (1) and (2).
Detection limit	A standard with a concentration of 0.005 mg/ml (0.1% of the test solution) should be tested and the spot visible.	The spot for the 0.1% solution was visible.
Robustness	The spots of the aged samples should compare with that of the freshly prepared sample with regards to appearance, intensity and position (Rf).	The spots obtained for the aged samples (after 48 hours) were comparable to those of the freshly prepared samples.
System suitability	The test is not valid unless the chromatogram obtained with standard solution (3) shows three spots which are clearly separated.	The chromatogram obtained with standard solution (3) showed three spots which were clearly separated.

[1] Solution (2) is not part of the monograph test, but was used in the validation to identify the spots for specificity.

[2] Solution (A): lumefantrine reference standard in solvent,

[3] Solution (B): analytical placebo in solvent.

The method for artemether related substances in artemether/lumefantrine tablets, as supplied by the WHO, was successfully adjusted and the validation thereof was presented in Appendix H. The validation results were summarised in Table 5.3. As this method was a limit detecting method, it would identify any of the artemether related substances present in a product, as well as semi-quantitatively (through visual comparison) evaluate whether it complied with the set specifications or not.

5.2.4 Assay

The proposed method in the artemether/lumefantrine tablets monograph (Ph.Int.) for the assay of artemether and lumefantrine comprises the HPLC method, as described in section 5.2.4.1.

5.2.4.1 High performance liquid chromatography (HPLC)

Whereas the TLC is defined as a planar technique, the Ph.Int. (2008) defines HPLC as follows:

> *"This column based chromatographic method is also known as high-performance or high-speed liquid chromatography. The HPLC technique mainly consists of an adsorbent packed into a column designed to withstand high pressures in order for the mobile phase to be pumped through the column at a high speed. These methods need specialised apparatus to deliver fast and efficient separations, suitable for precise quantitative measurements of components."*

The manufacturer's assay method, as supplied by the WHO, was selected and validated (Appendix I), following minor adjustments.

A switch in the wavelength (Table 5.4 and 5.5), from 210 nm to 380 nm after 28 minutes was investigated. The lumefantrine peak was smaller at 380 nm than at 210 nm, where it suppressed that of artemether in such a way that it was difficult to observe. As artemether does not absorb at 380 nm, 210 nm was required for the analysis of artemether.

Table 5.4 Chromatographic conditions and procedures of the HPLC method for the assay of artemether/lumefantrine tablets

HPLC assay method for artemether/lumefantrine tablets of a manufacturer, as supplied by the WHO	
Column:	15 cm x 3.9 mm C_{18} column, 5 µm (Symmetry is suitable)
Mobile Phase A:	Ion pair reagent : acetonitrile (700 : 300)
Mobile Phase B:	Ion pair reagent : acetonitrile (300 : 700)
Solvent:	Ion pair reagent : water : 1-propanol (200 : 60 : 200) diluted to 1000 ml with acetonitrile
Injection volume:	20 µl
Flow rate:	1.3 ml/min
Wavelength:	210 nm for the first 28 minutes and 380 nm from 29 to 55 minutes
Procedure:	Prepare solutions (1) containing 20 mg of artemether (about 120 mg of lumefantrine) of test sample in 100 ml (add approximately 85 ml of solvent, sonicate for 20 minutes, allow to cool to room temperature and dilute to volume), (2) accurately weigh 20 mg of artemether RS and 120 mg of lumefantrine RS in 100 ml (treat the same as solution (1)).
Ion pair reagent:	Dissolve 5.65 g of sodium hexanesulfonate and 2.75 g of sodium dihydrogen phosphate in about 900 ml of water. Adjust pH to 2.3 using phosphoric acid. Dilute to 1 000 ml and filter (0.45 µm).

Table 5.5 Gradient table for the HPLC assay test of artemether and lumefantrine in FDC products

Time (min)	**Mobile phase A (% v/v)**	**Mobile phase B (% v/v)**	**Comments**	**Detection (nm)**
0 - 28	60	40	Isocratic	210
28 - 29	60 to 0	40 to 100	Linear gradient	380
29 - 45	0	100	Isocratic	380
45 - 46	0 to 60	100 to 40	Return to initial composition	380
46 - 55	60	40	Isocratic re-equilibration	380

During revision by the expert committee, some concern was raised regarding the solubility of lumefantrine in the given solvent. An investigational experiment was conducted at 22 – 25°C, in order to verify the solubility of the lumefantrine standard.

Table 5.6 Solubility of the lumefantrine API in different solvent concentrations

Lumefantrine sample	Concentration (mg/100 ml) (% of target concentration)	Recovery (mg/100 ml) (% of target concentration)
1	121.2 (100%)	120.9 (100.3%)
2	181.0 (150%)	177.0 (146.7%)
3	240.7 (200%)	198.5 (164.9%)
4	480.7 (401%)	201.1 (167.8%)
5 (24 hours)	181.0 (150%)	181.6 (151.0%)
6 (24 hours)	240.7 (200%)	202.2 (168.0%)

A full recovery was obtained for concentrations of up to one and a half times (180 mg/100 ml) that of the target concentration (120 mg/100 ml), thus lumefantrine completely dissolved up to 180 mg/100 ml. Samples with higher lumefantrine concentrations (samples 3 and 4 in Figure 5.3 and Table 5.6) proved to be fully saturated at about 180 mg/100 ml. A maximum of 201.09 mg/100 ml was recovered with solutions containing up to 480.66 mg/100 ml. These over saturated samples formed a yellow sediment upon standing, hence supporting the solubility test results.

In order to evaluate the possibility of precipitation of lumefantrine samples 1 and 2 (Table 5.6), they were tested again after 24 hours of standing at ambient conditions. The results, as presented in Table 5.6 (samples 5 and 6), were within the limits for experimental error. This method had thus been proven suitable with regards to solubility, for samples containing up to about 180 mg of lumefantrine per 100 ml.

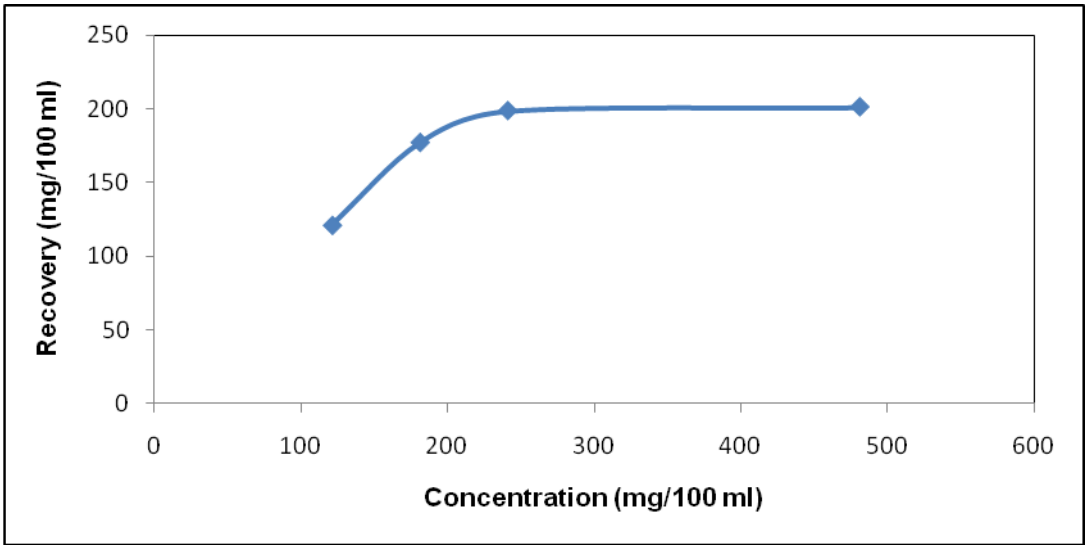


Figure 5.3 Recovery of lumefantrine at different solvent concentrations during solubility test.

The samples with concentrations of about 240 mg/100 ml did not completely dissolve, since a sediment formed at the bottom of the volumetric flask. In order to investigate the influence of changes to the solvent on the solubility of the actives, more 1-porpanol was added and the following results obtained. With the addition of 1-propanol (100 ml and 200 ml), the solubility did not improve, as demonstrated in Table 5.7, but continued to produce results with saturation at about 180 mg/100 ml. It was thus decided not to change the composition of the solvent in the method.

To prevent precipitation of lumefantrine from standard and sample solutions upon cooling, a note was included in the monograph that all prepared solutions should be kept at temperatures not below 20°C.

Table 5.7 Evaluation of the HPLC solvent regarding the solubility of lumefantrine after 24 hours of standing at ambient conditions

Sample	Concentration (mg/100 ml) (% of target)	Solvent	Recovery (mg/100 ml) (% of target)
1	121.01 (100%)	100 ml additional 1-propanol	118.03 (97.5%)
2	127.07 (100%)	200 ml additional 1-propanol	124.29 (97.8%)
3	245.74 (200%)	100 ml additional 1-propanol	187.27 (152.8%)
4	242.64 (200%)	200 ml additional 1-propanol	183.27 (151.1%)

The method was validated (Appendix I) in accordance with the ICH Q2(R1) (2005:1-13) guideline and the data summarised in Table 5.8.

Table 5.8 Summary of validation results for artemether/lumefantrine tablets HPLC assay method

Parameter	Acceptance criteria	Results
Specificity	The solvent and placebo solution should not generate any peaks that will interfere with the determination of the active ingredients (artemether and lumefantrine), any other peaks should be discernable from those of these two actives. For stressed samples no interference of the API peak should be detected.	Absence of interference was demonstrated for the solvent and the placebo solution. No interferences with the API peaks were detected after stress testing (peak purity measurement).
Linearity & range	The method should be linear over a range of 80 – 120% with the correlation coefficient (R^2): ≥ 0.99.	The method proved to be linear for a concentration range of 80 – 120%. The R^2 for artemether was 0.9979 and for lumefantrine 0.9981.
Accuracy	The difference between the percentage mean recovery and the theoretical (100%) must be ± 2.0% (Recovery should be 98.0 – 102.0%).	The mean recovery for artemether: 100.9% (100.5 – 101.3%). The mean recovery for lumefantrine: 100.1% (99.7 – 100.7%).
Precision		
A: Repeatability (spiked solutions)	Repeatability of analysis on six solutions at 100% of the target concentration (200 µg/ml artemether and 1200 µg/ml lumefantrine). %RSD ≤ 2.0%.	The mean for artemether: 100.9% and %RSD = 0.3%. The mean for lumefantrine: 100.1% and %RSD = 0.4%.
B: Reproducibility (tablets)	The %RSD obtained by each analyst should be ≤ 2.0%. The %RSD of the six preparations between different analysts must be ≤ 3.0%.	Mean (%RSD) artemether Analyst A: 95.7% (0.5%), Analyst B: 95.7% (1.8%). Mean (%RSD) lumefantrine Analyst A: 99.8% (0.5%), Analyst B: 96.8% (1.3%). %RSD for artemether = 1.2%. %RSD for lumefantrine = 1.9%.
Robustness	Poor chromatography due to a change in pH should lead to a note in the method. The peak areas of the aged solution should not differ from the initial solution with more than 2.0%.	The change in the pH value of the ion pair reagent in the mobile phase had an insignificant effect on the retention time. The peak areas of artemether differed with 2.0% from the initial in 24 hours and with 2.3% in 48 hours. The peak areas of lumefantrine differed with 0.5% from the initial in 48 hours.

This method proved to be specific for both the artemether and lumefantrine APIs. Linearity was established over a range of 80 – 120%. Accuracy was demonstrated, while precision testing proved the method to be repeatable and reproducible (in different laboratories). The method was robust with respect to pH changes of the ion pair reagent (mobile phase), while the outcomes showed that the column should be tested for its efficacy before use. A specific column was further advised in the monograph method, as summarised in Table 5.8.

In addition to the specificity tests, a stress study was performed on solutions containing artemether and lumefantrine (20/120 mg, as present in the tablet). This was done in order to evaluate any possible degradation and the influence thereof on the chromatography of artemether and lumefantrine in solution.

Artemether/lumefantrine stability under stress conditions

In order to evaluate the specificity and stability of artemether and lumefantrine, 20 mg of artemether and 120 mg of lumefantrine reference standards were co-dissolved in 100 ml of solvent, as for the HPLC method (Table 5.4). 10 ml of each of the samples was then further diluted (10 : 5) with different solutions (acidic, alkaline and oxidative) to create stress media.

The samples were stored at 20 – 25°C and analysed, using the HPLC assay method (section 5.2.4.1). The dilution solutions used were 0.1 N HCl (acidic), 0.1 N NaOH (alkaline), 3% of H_2O_2 (oxidative), and water. For each solvent all the injections done over time were from the same vial in an attempt to limit the variables.

Artemether was stable in H_2O_2 and water over a period of 24 hours, while degradation was observed in HCl (about 7%) and NaOH (about 4%) (Table 5.9). TLC testing for the artemether related substances confirmed the presence of degradation products in the HCl and NaOH solutions, while no evidence of any degradation products were found in the H_2O_2 and water samples.

Table 5.9 Artemether stability under acidic, alkaline and oxidative stress conditions

Time (hours)	Percentage recovery of artemether in various stress media			
	0.1 N HCl	**0.1 N NaOH**	**3% H_2O_2**	**H_2O**
0	101.0%	100.7%	100.2%	100.5%
4	98.6%	98.2%	100.6%	102.1%
8	98.4%	97.3%	101.1%	101.5%
12	97.1%	96.6%	100.2%	101.3%
16	95.1%	97.5%	101.8%	100.7%
20	93.0%	97.5%	101.8%	100.8%
24	93.6%	97.0%	100.8%	101.5%

Lumefantrine proved to be stable in acidic, oxidative and water media, with the percentage recovery of 100%, from the initial injection up to 24 hours, without any significant difference in its recovery over time (Table 5.10). As soon as the alkaline medium was added to the stock solution for the dilution, the lumefantrine in this solution produced a milky appearance, while forming a yellow sediment upon standing. The sample could not be further diluted, as it would affect the concentration of lumefantrine, as per the study described under section 4.2.6.1, since this concentration would be too low for the detection of artemether. The results revealed the same behaviour over a period of 24 hours, as the assay method detected only 3% in the initial sample and 1% after 24 hours. These results were supported by the visible precipitate that had formed in the vial after 24 hours.

Table 5.10 Lumefantrine stability under acidic, alkaline and oxidative stress conditions

Time (hours)	Percentage recovery of lumefantrine in various stress media			
	0.1 N HCl	**0.1 N NaOH**	**3% H_2O_2**	**H_2O**
0	99.9%	3.2%	100.9%	100.7%
4	100.0%	2.8%	101.1%	100.0%
8	100.2%	1.9%	101.3%	101.0%
12	100.2%	1.4%	101.5%	101.2%
16	100.4%	1.1%	101.6%	101.3%
20	100.4%	1.0%	101.7%	101.5%
24	100.5%	1.0%	101.8%	101.4%

Lumefantrine proved to be stable over a period of 24 hours in water, as well as under acidic and oxidative conditions. No conclusion could be made with regards to the alkaline media, since lumefantrine immediately precipitated upon mixing.

5.3 Specifications developed for the monograph of the artemether/lumefantrine powder for suspension

With regards to the Ph.Int. monograph of the artemether/lumefantrine powder for suspension, the same methods that had been developed and validated for the artemether/lumefantrine tablets, were used, after minor adjustments in sample preparation, where needed.

The components of "liquid preparations for oral use monographs" in the Ph.Int. (2008:5-360), as listed in Chapter 2 (Table 2.1), were used as a guideline for selecting the methods during the development of the monograph for artemether/lumefantrine powder for suspension.

The requirements for the monograph included the following (refer Table 2.1):

- Definition;
- Identity tests (TLC and HPLC);
- Artemether related substances (TLC); and
- Assay (HPLC).

5.3.1 Definition

The monograph for the artemether/lumefantrine oral suspension should comply with the monograph for "Liquid Preparations for Oral Use" in the Ph.Int. (2008) and the "Powders for oral solutions, oral suspensions and oral drops".

The definition, as per the monograph, was in accordance with the requirements generally set by die Ph.Int. (2008) for oral liquids:

> *"Artemether and lumefantrine oral suspension is a suspension of Artemether and Lumefantrine in a suitable vehicle; it may be flavoured. It is prepared by suspending the powder in the specified volume of the liquid stated on the label just before issue for use.*
>
> *The powder contains not less than 90.0% and not more than 110.0% of the amounts of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) stated on the label."*

5.3.2 Identification

For the identification of the artemether and lumefantrine APIs in the powder for suspension, the same TLC and HPLC methods developed and validated for the combination tablets, were used.

5.3.2.1 Thin layer chromatography (TLC)

The same TLC method, as developed (section 5.2.2.1) and validated (Appendix G) for the tablets, was adopted for use as an identification method for the monograph of the oral suspension. The sample preparation was adjusted in order to accommodate the difference in dosage form, whilst specificity (Figure 5.4) was performed in addition, to ensure that the method was not affected by the different excipients being present in the powder for suspension, especially the flavourants and antimicrobial preservatives.

Samples were prepared as described below and the procedure, as described in section 5.2.2.1, was followed.

For solution (A) shake a quantity of the powder, containing about 20 mg of artemether, for 5 minutes in 100 ml of solvent, filter and use the clear filtrate. For solution (B) dissolve 20 mg of artemether reference standard and a proportional quantity (according to the ratio in the powder) of lumefantrine reference standard in 100 ml of solvent.

Solutions containing the excipients[1] (according to the label of Co-artesiane®), did not produce any spots (Figure 5.4). The method could thus successfully be used for the identification of the APIs in the powder for suspension.

[1] The analytical placebo consisted of the following: saccharose (300 mg), Avicel (1,000 mg), citric acid (120 mg), xanthan gum (350 mg), methylparaben (70 mg), propylparaben (10 mg), coconut flavour (50 mg), silica anhydrous colloidal (50 mg).

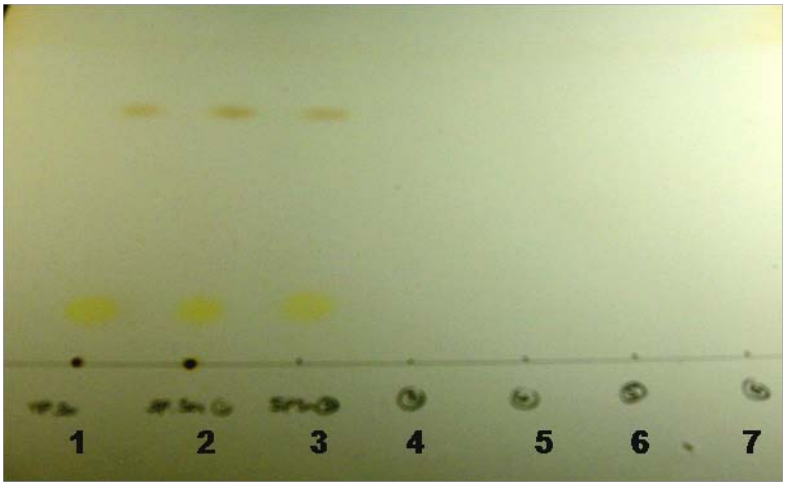


Figure 5.4 Artemether/lumefantrine powder for suspension identification through TLC (in daylight after treatment with *sulfuric/methanol TS*). Spot 1: artemether/lumefantrine powder for suspension, spot 2: artemether/lumefantrine tablet, spot 3: artemether and lumefantrine RS solution, spots 4 and 6: analytical placebo (powder for suspension), spots 5 and 7: analytical placebo (tablets).

5.3.2.2 High performance liquid chromatography (HPLC)

The HPLC assay method, as discussed in section 5.3.4.1, was also used for identification purposes.

5.3.3 Related substances

For the related substances of the artemether/lumefantrine powder for suspension, the same methods, as developed and validated for the artemether/lumefantrine tablets in section 5.2.3.1 (TLC artemether related substances), were used.

5.3.3.1 Thin layer chromatography (TLC) for artemether related substances

The sample was initially prepared in the same way as for the tablets, but it resulted in secondary spots being visible on the TLC test plate. Solutions of each excipient (as found on the product container), were prepared, following the procedure for sample preparation and TLC testing on the individual solutions. Saccharose, methylparaben and propylparaben produced spots visible under UV light at 254 nm and in daylight. The spots for obtained for methylparaben and propylparaben corresponded to the additional spots that had been observed with the initial sample preparation. The sample preparation was subsequently adjusted, by shaking a quantity of the powder,

containing 100 mg of artemether, with a mixture of 100 ml of water and 4 ml of *sodium hydroxide TS* (Ph.Int., 2008). Artemether and its related substances were immediately extracted from the sodium hydroxide phase with four times 25 ml quantities of dichloromethane, after which the combined extracts were evaporated to dryness. The residue was sonicated with 20 ml of solvent for 15 minutes, centrifuged and the clear supernatant used. This produced results in which no spots corresponded to either those of the excipients, nor of lumefantrine in the sample (Figure 5.5). This extraction with the sodium hydroxide solution was done to remove all the acidic and water soluble excipients, which for example included the acidic parabens.

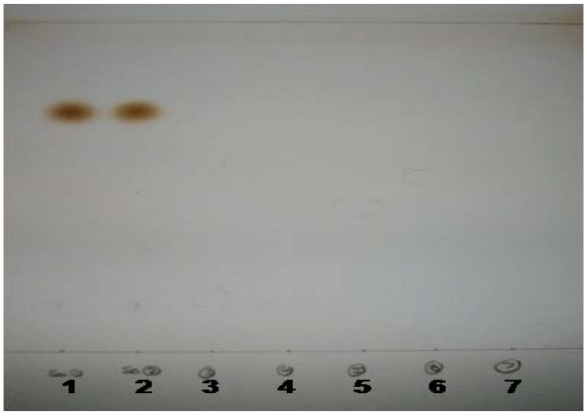


Figure 5.5 TLC plates for the artemether related substances limit test in a powder for suspension: Spot 1: artemether/lumefantrine standard, spot 2: artemether/lumefantrine powder for suspension, spot 3: saccharose, spot 4: citric acid, spot 5: methylparaben, spot 6: propylparaben and spot 7: benzoic acid.

5.3.4 Assay

The HPLC method that had been developed and validated (section 5.2.4.1) for the artemether/lumefantrine tablets, was used for the monograph of artemether/lumefantrine powder for suspension.

5.3.4.1 High performance liquid chromatography (HPLC)

The sample preparation in the assay method for the artemether/lumefantrine tablets was adjusted in order to accommodate the differences of the powder for suspension dosage form. In this section, the proposed change in sample preparation and specificity of the method are described.

Different sample preparations were investigated. After about 6 ml of the resuspended sample was weighed and treated with solvent, agglomerates formed in all samples.

Contrary, weighing of the powder and then treating it with the solvent did not result in any problems, hence the inclusion of this method in the final method, as described below.

Procedures adopted in the final method

> *"Weigh the contents of the container. To a quantity of the powder containing about 20 mg of artemether, accurately weighed, add 100 ml of solvent, sonicate for 20 minutes and allow to cool to room temperature. Filter through a 0.45 µm filter, discarding the first few ml of the filtrate. For solution (2) accurately weigh 20 mg of artemether RS and a proportional quantity (according to the ratio in the powder) of lumefantrine RS in a 100 ml volumetric flask. Add approximately 85 ml of solvent, sonicate until dissolved, allow to cool to room temperature and dilute to volume* (Ph.Int., 2008)*."*

Specificity

Samples were prepared in the same way as for the validation of the methods for artemether/lumefantrine tablets (Appendix I). A standard (B.6, Appendix I), sample and analytical placebo solutions were prepared, using the procedure for test solutions (B.5, Appendix I).

The measurements of the standards and the analytical placebo were carried out according to procedure B.7 (Appendix I). The chromatograms were examined for any additional peaks that may have interfered with the API peaks. The chromatograms in Figure 5.6 show the absence of any interference by the solvent and placebo.

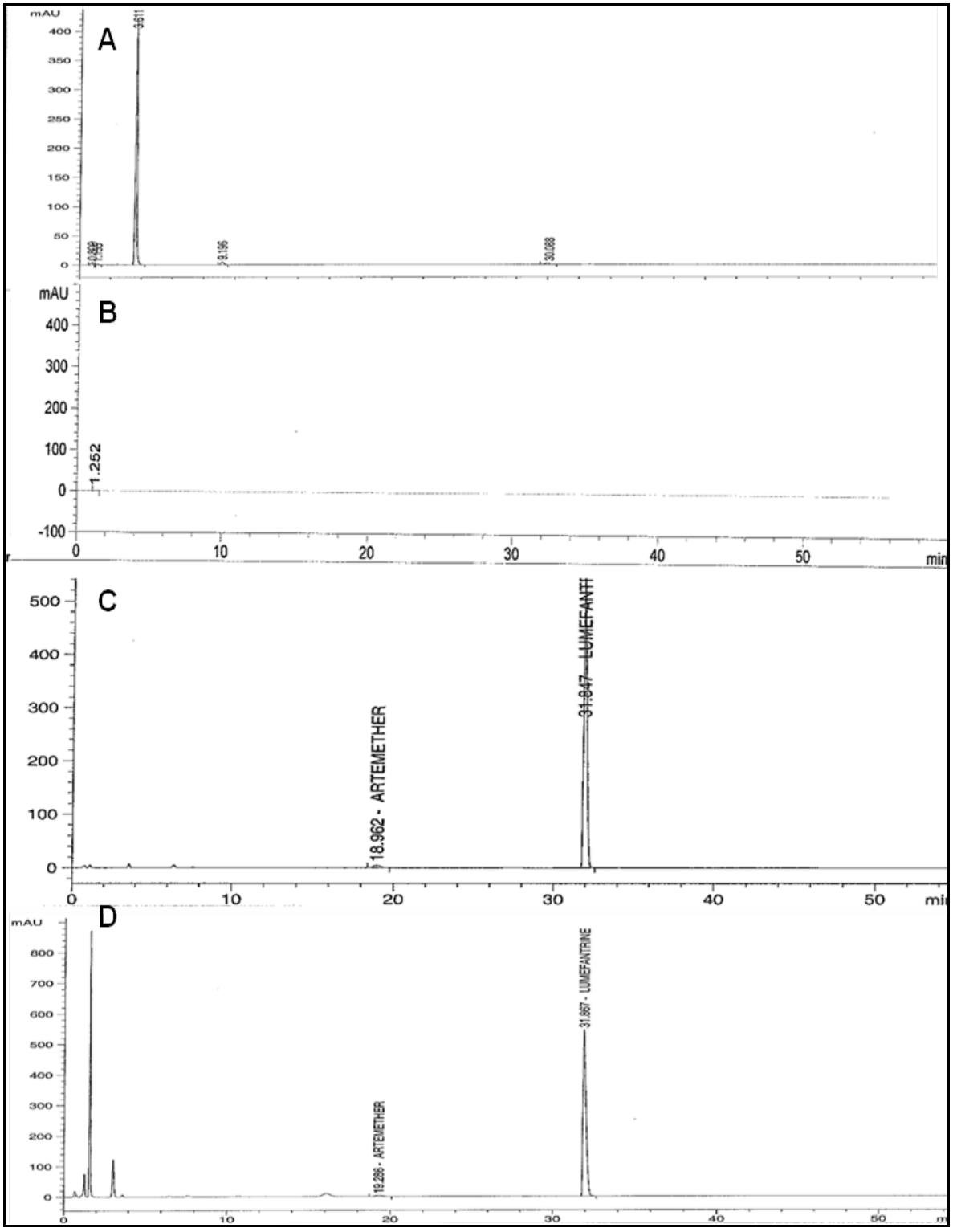


Figure 5.6 HPLC solvent chromatogram for artemether/lumefantrine powder for suspension A: placebo, B: solvent, C: standard solution and D: test solution.

5.4 Conclusion

This chapter outlined the general information relating to the artemether/lumefantrine tablets and artemether/lumefantrine oral solution (powder for suspension) monographs, and described the development of the methods, as defined under “Requirements” of these monographs.

For the identification test, a combination of a TLC and a HPLC method were used. A TLC method for the artemether related substances limit test was also developed. The APIs (artemether and lumefantrine) were simultaneously assayed, using the same chromatographic system (HPLC method). The main focus was to ensure effective and inexpensive methods, typically used in pharmacopoeial monographs, for testing of the artemether/lumefantrine products, in order to ensure products of suitable quality.

The validation of methods developed for the artemether/lumefantrine tablets, were summarised under the appendices. The TLC identification method can be located in Appendix G and that for artemether related substances in Appendix H. Appendix I presents the validation outcomes of the HPLC assay method.

The developed HPLC assay method included results of stress testing. These outcomes showed that artemether was stable in H_2O_2 and water for at least 24 hours, whereas degradation occurred in HCl (about 7%) and NaOH (about 4%) within 24 hours. Lumefantrine proved to be stable under acidic, oxidative and aqueous stress conditions for at least 24 hours. Although no conclusion under alkaline conditions could be made during this study, due to precipitation upon mixing, its stability in alkaline solution was already proven in section 4.2.6.1.

The methods developed for the artemether/lumefantrine tablets were for practical purposes verified for use on another dosage form, i.e. the artemether/lumefantrine powder for suspension. Sample preparations only required minor adjustments in order to accommodate the different dosage form. Specificity was also proven for each method.

The monographs that were adopted for inclusion in the Ph.Int., is included in Appendix B.

CHAPTER 6

Testing of commercial artemether/lumefantrine FDC samples

6.1 Introduction

The Research Institute for Industrial Pharmacy® (RIIP®), incorporating the Centre for Quality Assurance of Medicines® (CENQAM®), had tested artemether/lumefantrine FDC tablets sampled from different African countries by the WHO. As Quality Control (QC) manager at the RIIP®/CENQAM® at the time of testing I had the opportunity to be part of this project and subsequently incorporated the data generated for the statistical evaluation, as reported in this chapter.

The samples differed for example with regards to manufacturers, packaging and batch numbers. Since no monograph was available for the testing of these products in any of the pharmacopoeias from the ICH region (USP, BP and EP), it was decided to use the newly developed monograph (Chapter 5), included in the Ph.Int. This was an ideal opportunity to evaluate the suitability of the new monograph in a QC laboratory.

The artemether/lumefantrine FDC tablets were sampled from rural areas in six countries, i.e. Cameroon, Ethiopia, Ghana, Kenya, Nigeria and Tanzania. These products were submitted to uniformity of mass, identification, assay and related substance tests.

The aim of this project was:

1. To evaluate the method transfer results of selected methods of the newly developed monograph; and
2. To utilise the monograph for testing of commercially available samples.

6.2 Methodology

This section describes the methodology utilised during the method verification (section 6.2.1) and the statistical applications used to evaluate the data obtained during QC testing (section 6.2.2).

6.2.1 Method verification

The USP (2010) emphasises the importance for a QC laboratory to illustrate the suitability of a compendial method (i.e. method verification), used for testing under the actual conditions of the laboratory. A method verification was performed prior to testing of the commercial samples. For this method verification, the parameters, as summerised in Table 6.1, were evaluated.

Table 6.1 Summary of parameters tested during method verification

Tests	Method verification parameters
Appearance	N/A
Identification of APIs	N/A *
Related substances test: Identification of artemether related substances	System suitability Limit of detection
Assay of artemether and lumefantrine	System suitability Specificity Linearity and range Accuracy Precision (Repeatability)

* According to the (EDQM) (2005:5), no formal verification is required of methods for API identification tests in medicinal products, when utilising a compendial method.

Table 6.2 summarises the method verification protocol and acceptance criteria for the identification of artemether related substances, according to the test for related substances.

Table 6.2 Procedure and acceptance criteria utilised for method verification of the identification and limit of detection of artemether related substances, using TLC

Parameter	Description and acceptance criteria
System suitability	Carry out the test as described in section 1.14.1 Thin-layer chromatography (Ph.Int., 2008), using silica gel R5 as the coating substance and a mixture of 40 volumes of light petroleum R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 20 µl of a solution in water R : acetonitrile R (1 : 1), containing about 0.1 mg/ml, artemether, artenimol and α-artemether reference standards. After application, allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air, or in a current of cool air. Dip the plate in vanillin/sulfuric acid TS2. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight. **Acceptance criterion:** The test is invalid, unless the chromatogram obtained with this reference standard solution shows three clearly separated spots.
Limit of detection	Dilute 2 ml of the reference standard solution prepared for the system suitability (see above paragraph) to 20 ml with water R : acetonitrile R (1 : 1) and continue with the test, as described for the system suitability. **Acceptance criterion:** The spots in the chromatogram obtained with the 0.01 mg/ml (lowest limit*) solution should be clearly visible.

* According to the monograph, the different related substances have different limits. The concentration of this solution is equal to that of the related substance with the lowest limit in the monograph, therefore the concentration for some of the spots were lower than the specified limits.

The parameters for the verification of the assay method of artemether and lumefantrine were specificity, accuracy, precision (repeatability), linearity and range (EDQM, 2005:5). Table 6.3 summarises the method verification protocol and acceptance criteria for the assay method in the monograph. Test samples prepared as per Table 6.3 were analysed, using the assay method in the monograph for artemether/lumefantrine FDC tablets (Appendix I).

Table 6.3 Procedure and acceptance criteria utilised for method verification of the HPLC assay of artemether and lumefantrine in artemether/lumefantrine FDC tablets

Parameter	Description and acceptance criteria
Specificity	Prepare and inject a standard solution, containing about 200 µg/ml artemether and 1200 µg/ml of lumefantrine reference standards in the solvent specified in the method. Prepare and inject a sample solution, containing about 200 µg/ml artemether and 1200 µg/ml of lumefantrine in the solvent specified in the method. Inject the solvent to illustrate that solvent peaks do not cause any chromatographic interferences. **Acceptance criteria:** The peaks of the APIs in the sample solution should correspond to those represented by the APIs in the standard solution with regards to retention times and peak areas. The solvent should not present any peaks that may cause chromatographic interferences.
Linearity & Range	Prepare standard solutions with final concentrations of 50%, 100% and 150% (according to the laboratory's standard operating procedures (SOP)) of the target concentration (200 µg/ml for artemether and 1200 µg/ml for lumefantrine). In order to evaluate the linearity and range, inject the 50% solution in duplicate, the 100% solution five times and the 150% solution in duplicate. Determine the average chromatographic responses of each solution and plot the average system responses relative to the concentrations. Perform a least squares analysis/linear regression analysis and determine the correlation coefficient (R^2) and linear equation. **Acceptance criterion:** A plot of the average chromatographic responses *versus* concentrations should be linear over a range of 50 – 150%, with the correlation coefficient (R^2) ≥ 0.99.
Accuracy	Inject a standard solution having a concentration of 100% (200 µg/ml of artemether and 1200 µg/ml of lumefantrine) five times consecutively and calculate the percentage recovery of the system response (i.e. peak area) to evaluate the accuracy. **Acceptance criterion:** The difference between the percentage mean recovery and the theoretical concentration must be ± 2% for all five injections (recovery should be 98 – 102%).
Precision **Repeatability**	Use the results obtained for accuracy testing to calculate the %RSD of the system responses (i.e. peak area) of the specified samples and evaluate the repeatability. **Acceptance criterion:** %RSD ≤ 2%.

6.2.2 Statistical applications used to evaluate data

The data generated from the analyses during this study were evaluated, using the following statistical calculations and/or models:

6.2.2.1 Mean ($\bar{x}$)

$$\bar{x} = \frac{\sum_{i=1}^{N} Xi}{N}$$

For a data set, the mean (average) is the sum of the values (*Xi*) divided by the population size (N). Outlier values may influence the accuracy of the mean significantly, necessitating the calculation of the standard deviation of the population and the identification of potential outliers (Bolton, 1997:20).

6.2.2.2 Median (me)

$$me = \left(\frac{N+1}{2}\right)$$

The median is the value exactly in the middle of an ordered population, which divides the data population in half (Steyn *et al.,* 2003:103).

6.2.2.3 Standard deviation (s)

$$s = \sqrt{\frac{\sum(X-\bar{X})^2}{N-1}}$$

The standard deviation shows the variation from the mean value (**$\bar{x}$**). A low value indicates a narrow distribution, while a high value indicates data points that are spread out over a wide range, which may be attributed to the presence of outliers in the population. Outliers may be detected using whisker-box plots (Bolton, 1997:20).

6.2.2.4 Range

The difference between the largest and smallest values in a population is defined as the range of the population. It is calculated by subtracting the smallest value (population minimum) from the greatest value (population maximum) and provides an indication of statistical dispersion (Steyn *et al.,* 2003:124-125). Theoretically, if the range values are smaller or equal to the difference of the maximum and minimum of

the specifications (i.e. range of the specifications), all the samples within the population complies with the specifications of the specific QC-test.

6.2.2.5 Frequency distribution

A frequency distribution illustrates the distribution (number of occurrences) of data in a population (Bolton, 1997:5). The frequency distribution may be presented in a variety of graphs, including histograms, line graphs, bar charts and pie charts. Frequency distributions may be used for both qualitative and quantitative data (Steyn *et al.,* 2003:62-84).

6.2.2.6 Cumulative frequency

Cumulative frequency indicates the amount of values per class in a cumulative order to finally represent the total population (Bolton, 1997:11). Frequency analysis provides insight into how often a certain feature occurs, for example the number of samples in a population which have passed/failed a certain specification.

6.2.2.7 Histogram

A histogram is defined as a graphic illustration of the distribution of experimental data to easily compare data and distributions visually. The total area of the histogram is equal to the population size. A histogram may also display relative frequencies, in which case it illustrates the proportion of the population that falls into a specific group. This proportion will determine the skewness (Bolton, 1997:41; Steyn *et al.,* 2003:63).

6.2.2.8 Skewness

Skewness illustrates the distribution of the values as a measure of the asymmetry of the distribution (Figure 6.1). The skewness value can be positive or negative, or even undefined (Steyn *et al.,* 2003:98). A negative skew indicates that the number of values on the left side of the mean is lower than on the right side and more of the values (including the median) lie to the right of the mean, and *vica versa* for a positive skew. A zero value indicates that the values are relatively evenly distributed on both sides of the mean. A negative skew with a high mean will thus indicate the presence of higher values in the population.

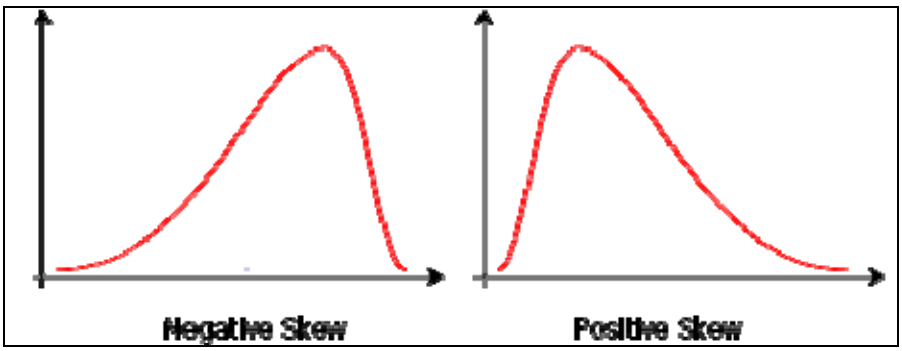


Figure 6.1 An illustration of negative and positive skewness (Anon 1, 2010).

6.2.2.9 Box and whisker plot

A box and whisker plot (Figure 6.2) is a graphical representation of numerical data through their five-number descriptors, i.e. the population minimum (lower extreme), lower quartile (Q1), median (Q2), upper quartile (Q3) and population maximum (upper extreme). A box and whisker plot may indicate values considered being outliers with regards to the specification of the quantiles, for example, setting the quantiles according to a specific value/specification may indicate out of specification results (Steyn *et al.,* 2003:155-156).

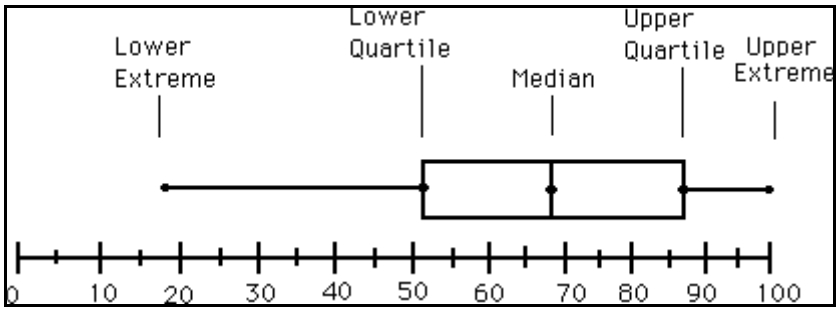


Figure 6.2 Example of a box and whisker plot (Anon 2, 2010).

6.2.2.10 Quartile

A quartile is a quantile that represents 25% of the distributed sample (Figure 6.2). The first (lower) quartile (Q_1) represents the cut off for the lowest 25% of the population. The second quartile (Q_2) divides the data set/population in half. The third (upper) quartile (Q_3) cuts off the highest 25% of the population (Steyn *et al.,* 2003:107-113). The position of the quartiles may be used to evaluate the distribution of the data in the population.

The data generated during this study was evaluated, using Statistica Version 9 (statSoft inc., Tulsa, USA).

6.3 Results and discussion

This section presents the results and evaluation of the method verification, as performed according to the protocol in section 6.2.1. The results obtained for the method verification were compared to the corresponding validation parameters results (Chapter 5), in order to evaluate the effectiveness of the method transfer.

6.3.1 Transfer verification of the qualitative identification of artemether related substances, according to the related substances test

The method verification performed on artemether/lumefantrine FDC tablets for the qualitative identification of artemether related substances, using the related substances test with TLC, illustrated good transferability, as summarised in Table 6.4.

Table 6.4 Results for the validation and verification of the method for the identification of artemether related substances

Parameter	Specification	Validation results	Verification results
System suitability	The test is invalid, unless the chromatogram obtained with the solution shows three clearly separated spots.	The chromatogram obtained produced three clearly separated spots.	The chromatogram obtained produced three clearly separated spots.
Limit of detection	The spots in the chromatogram obtained with the 0.01 mg/ml (lowest limit) solution should be clearly visible.	The 0.01 mg/ml spot obtained in the chromatogram was clearly visible.	The 0.01 mg/ml spot obtained in the chromatogram was clearly visible.

The validation, as well as the verification tests produced chromatograms in which the three spots were clearly separated and the lower limit (0.01 mg/ml) was clearly detectable (Table 6.4). The method was thus successfully transferred and could be used without any need for change.

6.3.2 Transfer verification of the quantitative assay method for artemether and lumefantrine

The results for the validation and verification of this method are summarised in Table 6.5.

The specificity results for both the validation and verification tests complied with the set specifications, thus both APIs were identifiable and no interferences were observed from the solvent used.

The percentage recovery (accuracy) for the method transfer verification correlated well with that obtained in the validation test (difference for artemether = 0.4% and for lumefantrine = 0.3%).

Linearity was illustrated in the range of 50 – 150% (according to the laboratory's SOP) of the target concentration for artemether (200 µg/ml) and for lumefantrine (1200 µg/ml) (R^2 = 0.99 for both APIs) and illustrated linearity over an even wider range than that of the validation (R^2 = 0.99 for both APIs from 80 – 120%).

The %RSD for five samples (repeatability) with a 100% concentration was 0.2% (artemether) and 0.1% (lumefantrine), with the verification correlating with the %RSD (0.3% for artemether and 0.4% for lumefantrine) obtained in the validation. It could thus be concluded that the QC laboratory was capable of executing this method successfully.

The method validation and verification results (Tables 6.4 and 6.5) were comparable. The method was therefore suitably transferred without any need for adjustments.

After verification of the methods, the test samples were submitted for testing.

Table 6.5 Results for the method validation and verification of the artemether and lumefantrine assay method

Parameter	Specification	Validation results	Verification results
Specificity	The retention times of the APIs in the sample should correspond to those represented by the APIs in the standard solution. The solvent should not present any peaks that may cause chromatographic interferences.	The retention times of the APIs in the sample corresponded to those of the APIs in the standard solution. The solvent did not present any interference.	The retention times of the APIs in the sample corresponded to those of the APIs in the standard solution. The solvent did not present any interference.
Accuracy	The difference between the percentage mean recovery and the theoretical recovery (100%) must be ± 2% for all five injections (recovery should be 98 – 102%).	The average percentage recovery for artemether = 100.9% (recovery range = 100.5 – 101.3%) and for lumefantrine = 100.1% (recovery range = 99.7 – 100.7%).	The average percentage recovery for artemether = 100.5% (recovery range = 100.3 – 100.8%) and for lumefantrine = 99.8% (recovery range = 99.2 – 100.1%).
Linearity & Range	The method should illustrate linearity from at least 80 – 120% of a 20 mg/tablet (200 µg/ml) for artemether and a 120 mg/tablet (1200 µg/ml) for lumefantrine. Correlation coefficient (R^2) ≥ 0.99.	Linear from 80% - 120% R^2 = 0.997 (artemether) R^2 = 0.998 (lumefantrine)	Linear from 50% – 150% R^2 = 0.999 (artemether) R^2 = 0.998 (lumefantrine)
Precision **Repeatability**	%RSD of the peak areas of five injections at 100% of the target concentration (200 µg/ml of artemether and 1200 µg/ml of lumefantrine) ≤ 2%.	%RSD = 0.3% (artemether) %RSD = 0.4% (lumefantrine)	%RSD = 0.2% (artemether) %RSD = 0.1% (lumefantrine)

6.3.3 Quality control test results for FDC tablets

No problems were experienced during testing of the artemether/lumefantrine FDC samples, using the monograph test methods. Out of specification (OOS) results were confirmed by retesting according to a Good Manufacturing Practice (GMP) procedure, i.e. the laboratory's Analytical Investigation (AI) procedure. A total of 108 products (N_{TOTAL}) were sampled by the WHO (Figure 6.3).

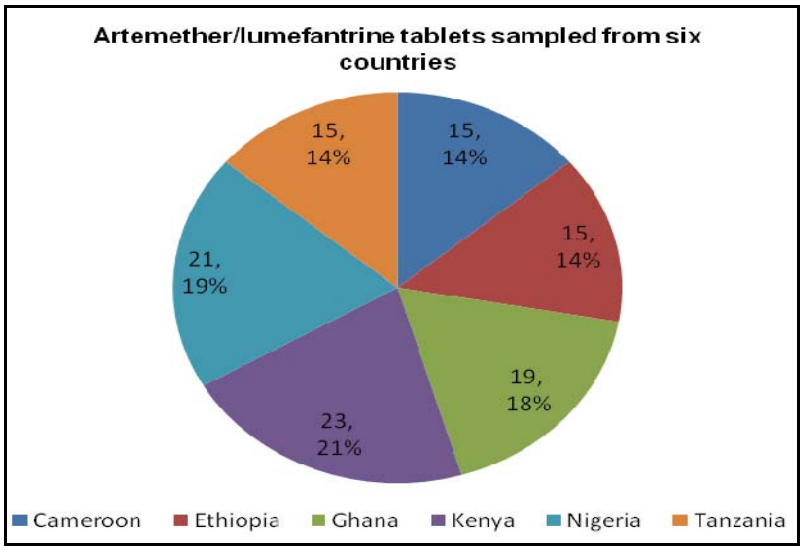


Figure 6.3 Number of batches sampled and tested from each country (percentage (%) of N_{TOTAL}).

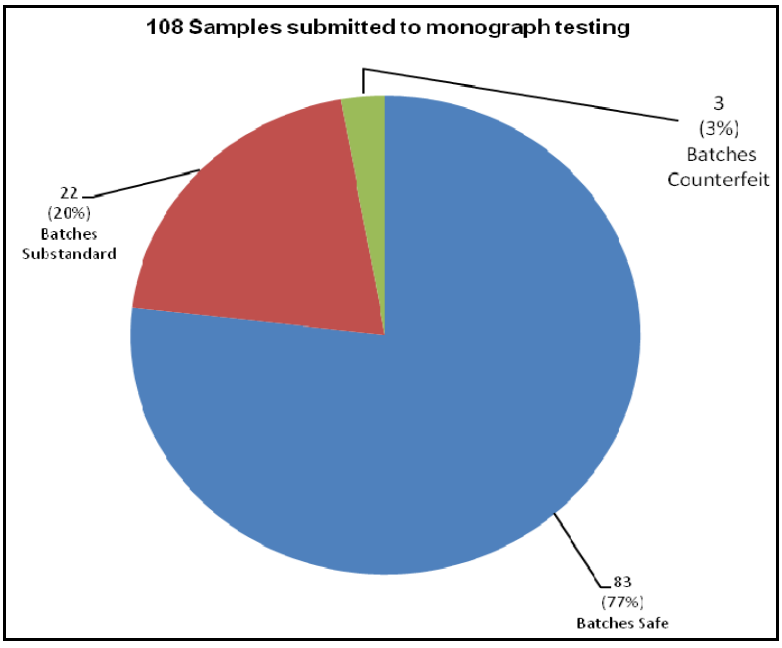


Figure 6.4 Compliance of the 108 batches (N_{TOTAL}) of artemether/lumefantrine 20/120 mg tablets, submitted for testing.

Eighty-three batches passed all specifications and could be considered as safe and of good quality. Three batches were classified as counterfeit, based on the fact that they either did not contain any active ingredients, or contained an incorrect amount of active ingredient, or an incorrect ingredient, and/or unapproved labeling and packaging (Ziance, 2008:71). Two of the batches tested produced no HPLC signals for artemether and another one contained only 28% of artemether. Visible tabletting errors were detected on all three these batches (Figure 6.4), as discussed below.

Twenty-two batches were identified as substandard, due to the failure of one or more of the following tests performed, i.e. visual, assay of APIs, or artemether related substances testing. Four of these failed both artemether and lumefantrine assays and another three failed assay testing for one of the two APIs (Table 6.6 and Figure 6.5). One batch failed the related substances testing.

For samples to be included in further evaluation (N_{VALID}), (i) the identification of both APIs (artemether and lumefantrine) had to be positive, (ii) the product description should have complied with the description on the package information leaflet (PIL), if included, and (iii) should not suggest it being a counterfeit, based on the visual appearance of the product or packaging (Chapter 1, section 1.6). The discussion below refers to the results, as summarised in Table 6.6.

Of the fifteen batches sampled in Cameroon, thirteen complied with all the specifications, whilst two batches showed clear tabletting errors (uneven surface with powder and/or crystals on surface). One of these two failed the artemether assay, while the other passed all other analytical tests.

Ten of the fifteen batches sampled in Ethiopia passed all the acceptance criteria with regards to all specifications. Five batches showed tabletting errors (light spots on surface, malformed engraving, crystals on surface).

In Ghana nineteen batches were sampled. One batch was suspected of being counterfeited (see identification below). Twelve batches complied with all of the specifications. One batch failed the lumefantrine assay, whilst another failed both artemether and lumefantrine assays. Four batches had visual problems (mottled tablets, black and brown spots on tablets, chipped tablets, score-line variation, poor quality logo, uneven coating and no inscription, in contrast with the information in the PIL). They, however, complied with the other parameters.

In Kenya twenty-three batches were sampled, two of which showed clear tabletting errors (uneven surface with crystals and spots), although they complied with the other

specifications. Another batch failed the related substance test, but also complied with all other specifications. Twenty of the batches tested passed all the tests.

Twenty-one batches were sampled in Nigeria. Two batches were excluded from testing, due to them possibly being counterfeit (see identification below) samples, while thirteen complied with all the specifications. The other batches showed the following results: One batch failed the lumefantrine assay while three other batches failed both lumefantrine and artemether assays. One of these three batches also failed the visual examination (tabletting errors, such as chipping, uneven coating and colour). One other batch failed only the visual examination.

All fifteen batches sampled from Tanzania complied with the monograph specifications.

Table 6.6 Summary of the test outcomes for 108 batches of artemether/lumefantrine FDC tablets, sampled by the WHO in six African countries

Country	Total number of batches tested		Visual appearance/ packaging	Related substances		Assay			
						Artemether		Lumefantrine	
	Tested	Passed		P	F	P	F	P	F
Cameroon	15	13	2 – uneven surface, powder & crystals on surface	15	-	14	1	15	-
Ethiopia	15	10	5 – light spots, malformed engraving, crystals on surface	15	-	15	-	15	-
Ghana	19	12	1 – suspected counterfeit; 4 – mottled, chipped tablets with spots, uneven coating, poor quality logo & no inscription.	19	-	18	1	17	2
Kenya	23	20	2 – uneven surface with crystals & spots	22	1	23	-	23	-
Nigeria	21	13	2 – suspected counterfeit; 2 – chipping, uneven coating & colour	21	-	18	3	17	4
Tanzania	15	15	-	15	-	15	-	15	-

(P = Number of batches passed, F = Number of batches failed).

6.3.3.1 Appearance

All batches were visually inspected according to the manufacturer's description (PIL) with regards to appearances. Samples for which specifications were unavailable were visually inspected for obvious tabletting errors, e.g. chipping and crystals on the surface. In total, eighteen (17%) of the batches tested did not comply with the required/expected appearances and/or displayed a variety of tabletting errors, including uneven surfaces, crystals on the surface, mottled and chipped tablets, malformed engravings, inferior quality logo's and no inscriptions where specified.

6.3.3.2 Identification

All samples were submitted to HPLC identification testing, as described in the monograph for artemether/lumefantrine FDC tablets (Appendix G). Three batches generated a negative result for the identification of artemether. The method thus positively identified these batches as counterfeit (according to the definition applicable to this study, section 1.6).

6.3.3.3 Assay

The assay for artemether and lumefantrine produced the results summarised in Table 6.7.

Table 6.7 Descriptive statistics table of artemether and lumefantrine assay results

API	Descriptive statistics						
	N_{VALID}	Mean Assay	Minimum Assay	Maximum Assay	Range Assay	Standard Deviation	%RSD
Artemether	105	95.5%	83.7%	104.6%	20.9%	3.5%	3.6%
Lumefantrine	105	94.8%	71.0%	101.1%	30.1%	4.0%	4.2%

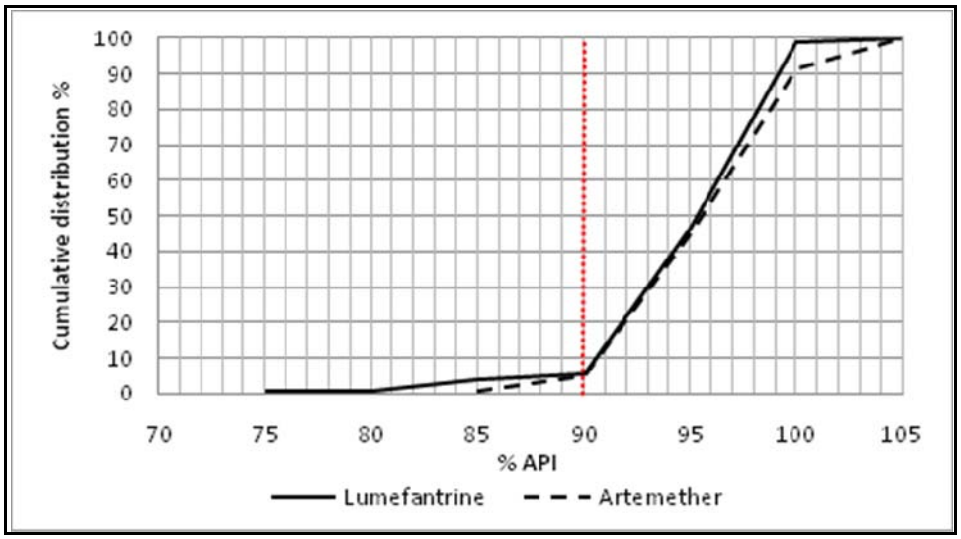


Figure 6.5 Cumulative distribution of artemether and lumefantrine assay results.

The mean assay result for artemether was 95.5% and for lumefantrine 94.8%, thus complying with the specifications of 90.0 – 110.0%. The maximum assay result for artemether (104.6%) and for lumefantrine (101.1%) were both within the upper limit of 110.0%, whereas the minimum assay results of 83.7% for artemether and 71.0% for lumefantrine indicated that some batches were below the lower limit of 90.0% (Table 6.7, Figure 6.5). Figure 6.5 clearly illustrates how the number of failing samples, i.e. the four batches failing both APIs and the other three failing only one of the two APIs, significantly influence the distribution of the results, as seen in the curve.

In order to evaluate the distribution of the assay values, all the samples that were within specification (90.0 – 110.0%) were used in the statistical applications (Figures 6.6 to 6.9). The box and whisker plot for artemether (Figure 6.6) illustrated the lower quartile (93.9%), median (95.6%), upper quartile (97.7%), and the %RSD between the hundred samples (within specification for artemether) being 3.1%. The skewness was determined as 0.5, while the standard deviation was 2.9% and the variance 8.6%. This illustrated an even distribution of results.

The artemether/lumefantrine FDC products tested contained only 20 mg of artemether, resulting in a small HPLC response peak, which may have attributed to the higher dispersion of the data. The box and whisker plot of lumefantrine (Figure 6.8) illustrated the lower quartile (94.1%), median (95.3%) and the upper quartile (96.9%). The standard deviation of ninety-nine samples (which complied with the specifications) was 2.3%, the skewness only 0.1 and the variance 4.7%. These all illustrated a close distribution of the results.

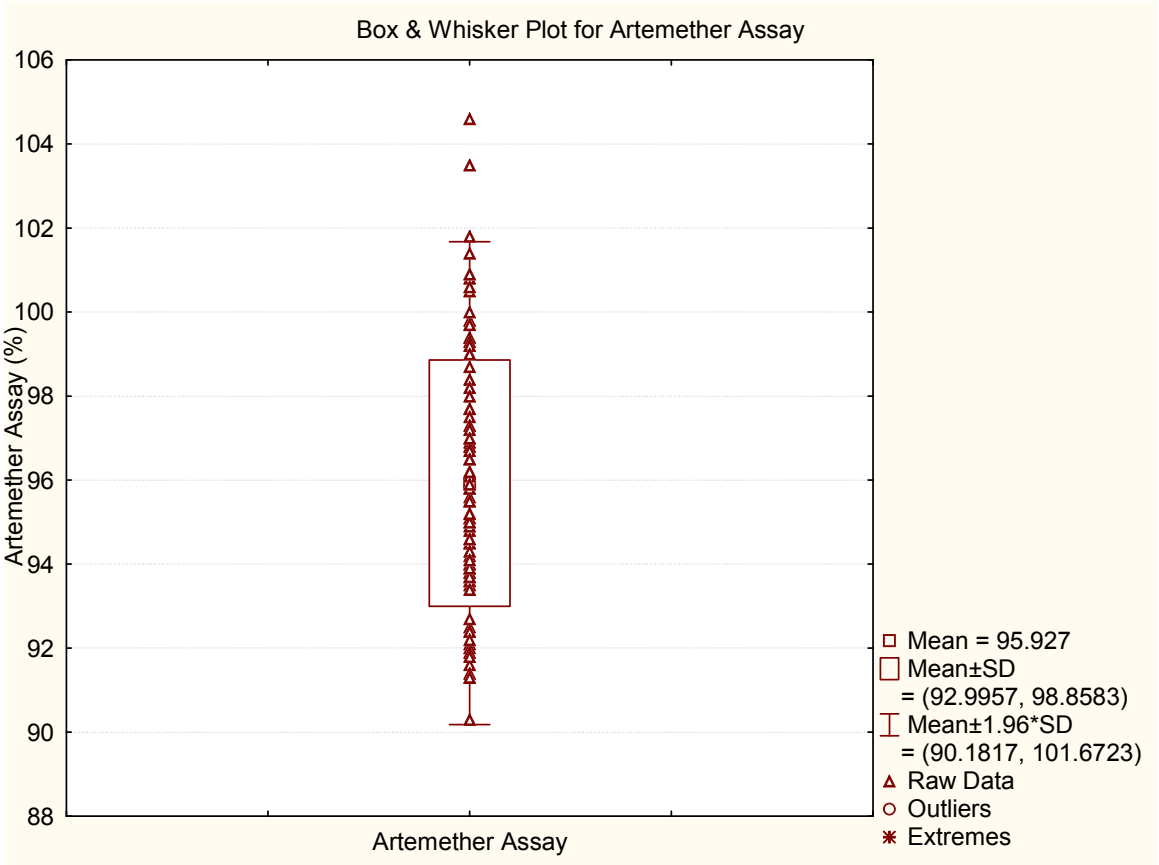


Figure 6.6 The box and whisker plot for artemether assay results.

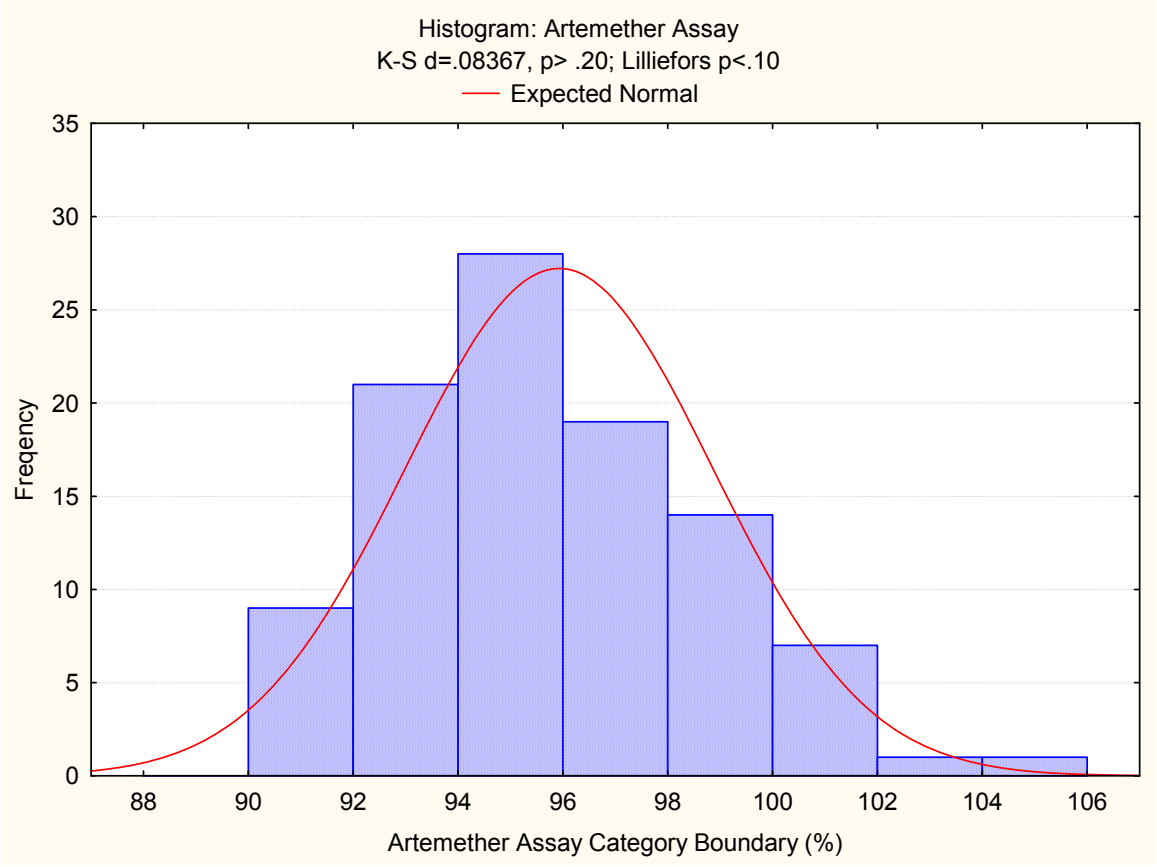


Figure 6.7 Histogram for artemether assay results.

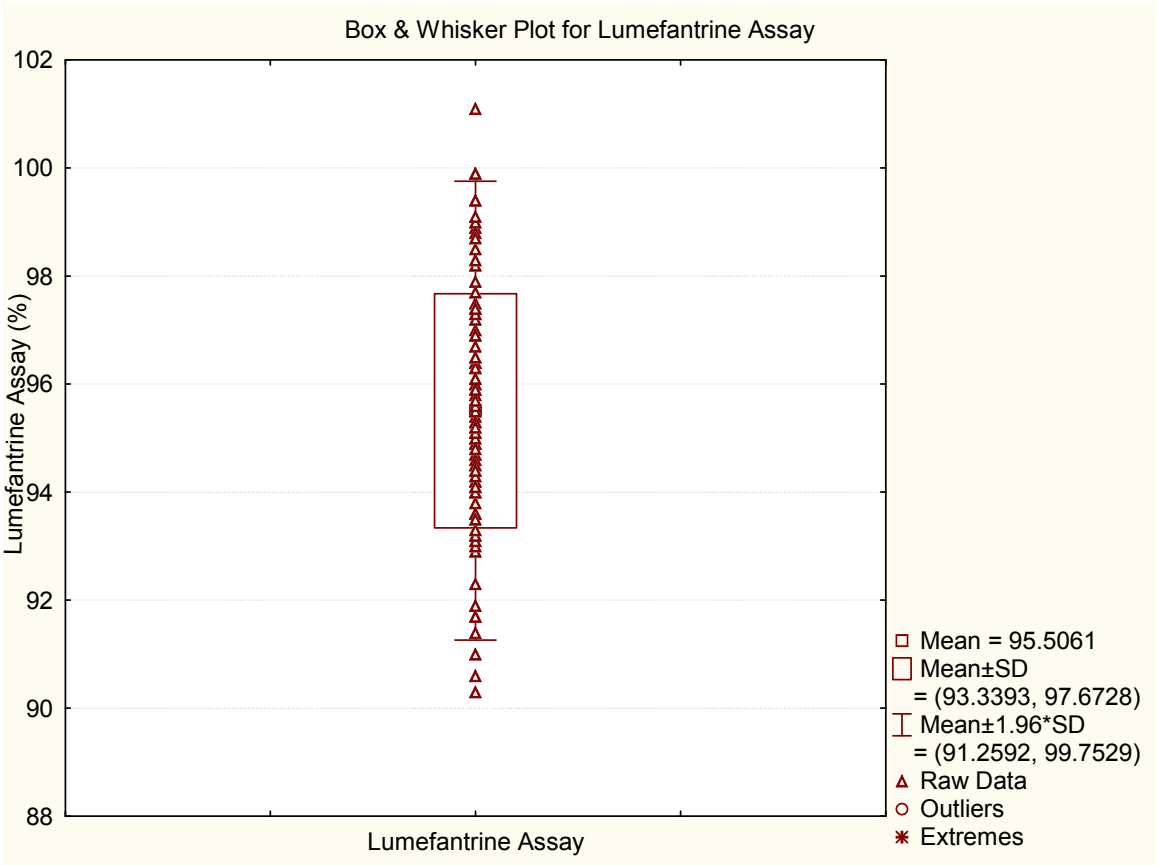


Figure 6.8 The box and whisker plot for lumefantrine assay results.

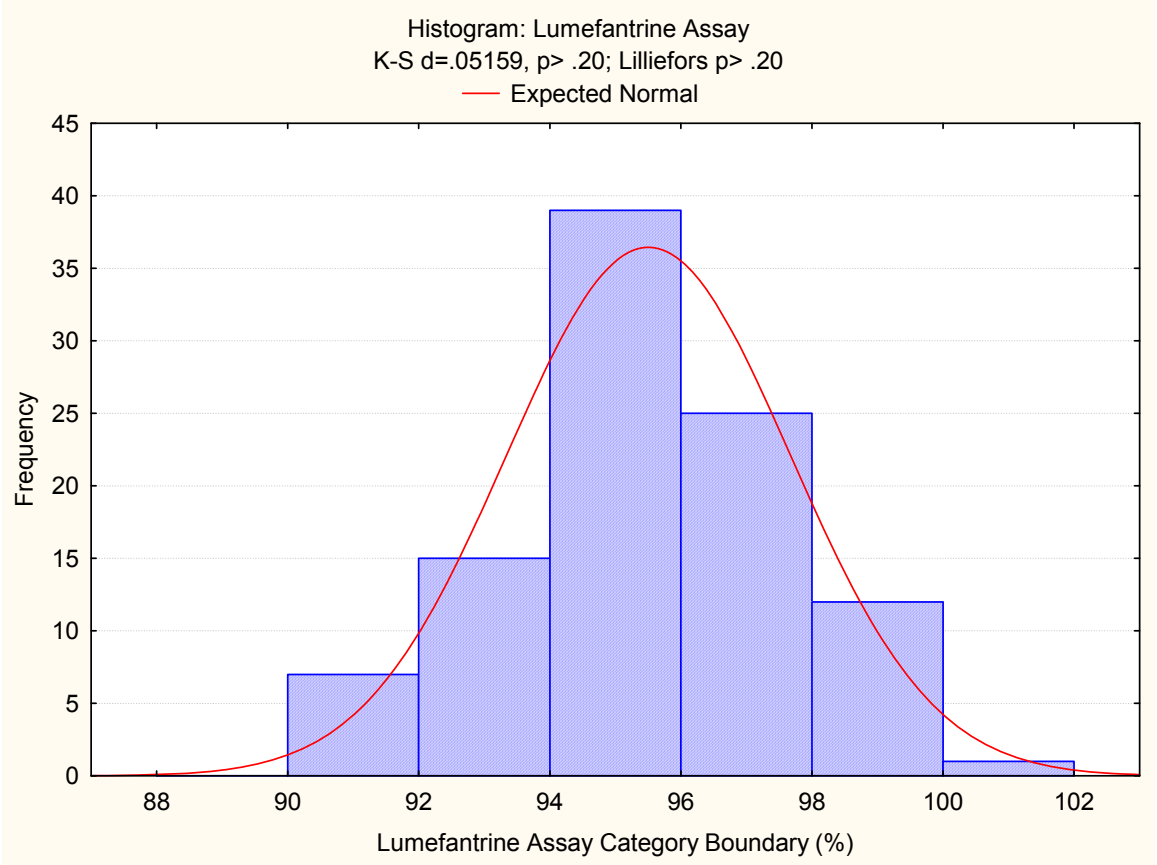


Figure 6.9 The histogram for lumefantrine assay results.

6.3.3.4 Related substances

All commercial batches were submitted to the test for related substances of artemether, as described in the monograph for artemether/lumefantrine tablets (Appendix H). The TLC chromatograms of some of the samples tested revealed spots which corresponded in position (R_f) to those of the impurities of artemether (impurities A, B, C and D). Only one batch (1%) did not comply with the monograph specification, i.e. the intensities of the impurity spots were higher than that of the reference impurity solution.

6.4 Conclusion

The method verification tests performed for transferability of the monograph methods, produced results concordant with, or better than that obtained during the validation process. It was hence concluded that the method was successfully transferred without any need for changes. According to the results, it was clear that these methods could easily be transferred to different QC laboratory environments to produce accurate results.

Three (3%) of the batches tested did not comply with the N_{VALID} parameters after visual inspection and the HPLC identification testing (no positive identification for both APIs as required) was thus classified as $N_{INVALID}$ and therefore excluded from further statistical evaluation, due to possible counterfeit samples. $N_{TOTAL} = N_{VALID} + N_{INVALID}$ in this case 108 = 105 + 3.

$N_{VALID} = N_{STANDARD} + N_{SUBSTANDARD}$. Substandard products ($N_{SUBSTANDARD}$), as discussed in Chapter 1, are samples that test positive for both APIs, but fail one or more of the other tests according to the monograph test specifications; in this case assay, related substances, as well as visual inspection. In this study, about 8% of the samples failed the assay for both APIs, 20% (22 batches $N_{SUBSTANDARD}$) could be identified as substandard by failure of one or more tests.

Eighty-three batches (77%, $N_{STANDARD}$) complied with all the specifications of the lumefantrine monograph (as developed in Chapter 5), as well as visual testing and could therefore be classified as products of acceptable quality.

It could thus be concluded that the methods in the monograph for artemether/lumefantrine FDC tablets were able to distinguish between acceptable quality, substandard and counterfeit products, and if used for testing, would assure the release and distribution of FDCs of acceptable quality.

CHAPTER 7

Summary and Conclusion

7.1 Introduction

With almost half of the world's population at risk of contracting malaria, a disease that currently is responsible for the deaths of about 10 000 pregnant women and of up to 200 000 infants annually, just in Africa (WHO, 2009), it is certainly one of the biggest health challenges our global society faces.

The World Health Organization (WHO), together with several other organisations, are continuously working towards improved prevention, earlier and more accurate diagnosis, as well as better availability of effective and affordable treatments for patients diagnosed with malaria.

Artemether and lumefantrine tablets (Coartem®) were recently (2009) approved for the treatment of acute and uncomplicated malaria in children and adults by the United States Food and Drug Association (US FDA, 2009), and forms part of the current, most effective and less expensive treatment regimes against malaria. With no existing monographs for either the lumefantrine API, nor the artemether/lumefantrine fixed-dosage-combination (FDC) products in the Ph.Int., the development of these monographs has become a high priority to the WHO.

To pursue the set study objectives, a systematic research approach was followed, as illustrated in Figure 7.1. The approach consisted of three stages, namely:

- **STAGE 1:** Evaluation of the solid-state properties of the artemether and lumefantrine APIs and the development of a monograph for the lumefantrine API;
- **STAGE 2:** Development of monographs for the artemether/lumefantrine dosage forms (tablets and powder for oral suspensions); and
- **STAGE 3:** Assessment and application of the newly developed monographs.

Methods were developed for possible inclusion in the Ph.Int. monographs, in accordance with the WHO specifications and were validated, compliant with ICH guideline Q2(R1) (2005:1-13).

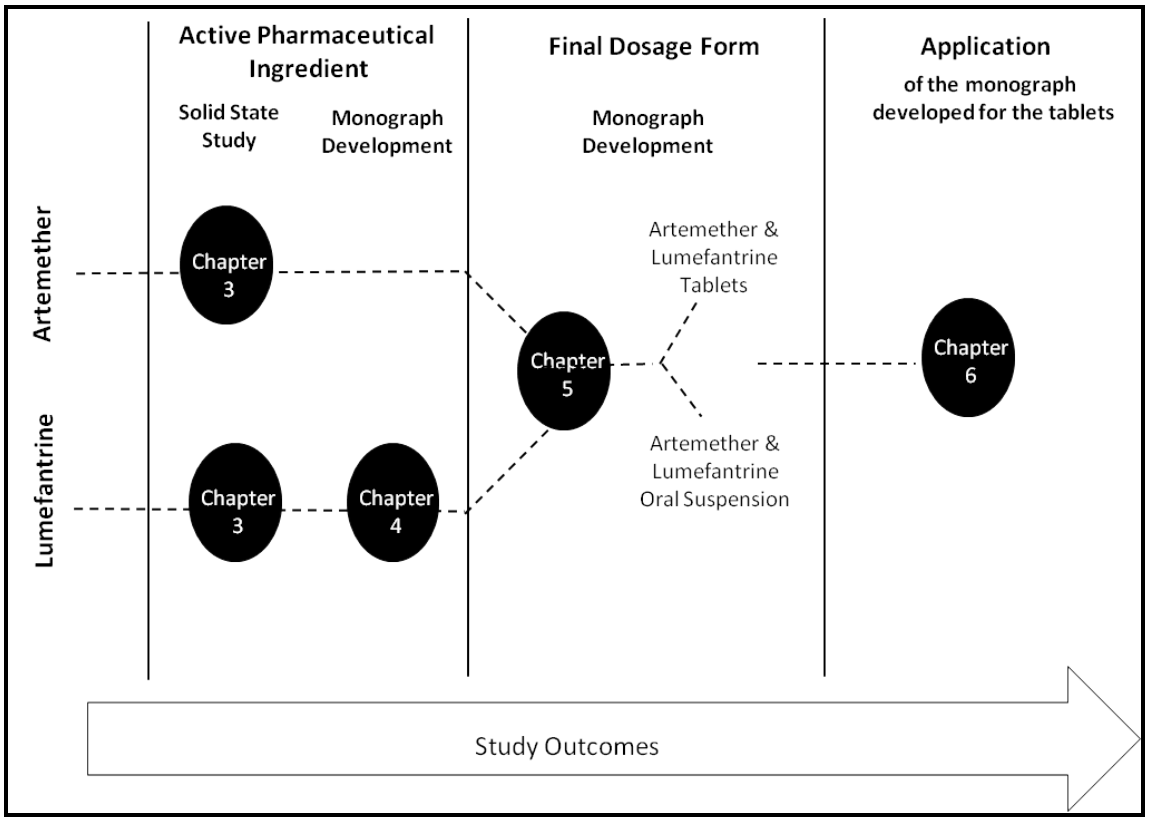


Figure 7.1 Schematical representation of the methodology followed and of the achieved outcomes of this study.

7.2 STAGE 1: Evaluation of the solid-state properties of artemether and lumefantrine APIs and the development of a monograph for the lumefantrine API

For the development of the lumefantrine API and fixed-dosage-combination (FDC) monographs, it was important to investigate whether different polymorphic forms existed, and if so, to characterise them (Chapter 3).

With regards to artemether, the outcomes of all the physico-chemical analyses during polymorphic screening consistently confirmed only one form, similar to that of the raw material tested.

Although the polymorphic screening of lumefantrine revealed no polymorphic forms, it was found that the XRPD patterns were influenced by preferred orientation and that the melting points reported correlated well with the differences observed in particle size and/or morphology. This finding emphasised the importance for manufacturers of lumefantrine to consider the possibility that the melting point may be influenced by

crystal habit and particle size, and would it be advisable not to characterise and identify lumefantrine based upon melting point.

The results from the physico-chemical analyses during the polymorphic screening of artemether and lumefantrine APIs revealed that only one crystal packing for each API existed, i.e. a potentially, thermodynamically, stable form. This observation suggested a reduced risk, compared to using a metastable form during manufacturing that may undergo polymorphic transformation into the potentially, thermodynamically, stable form, with significantly different physico-chemical properties, compared to the metastable form.

Based upon the above observations, it could be concluded that no reference with respect to the existence of polymorphism was required under the *Additional Information* section of the Ph.Int. monograph.

In Chapter 4, the developed methods for the lumefantrine API monograph were presented. Figure 7.2 summarises all the methods developed and lists the validated parameters. The selected identification tests comprised either a standalone IR method, or alternatively a combination of a UV-VIS and a TLC method. The standard pharmacopoeial methods for heavy metals, sulfated ash and loss on drying were proposed and agreed to. For the determination of the related substances, an HPLC and a newly developed TLC method were included, allowing for a choice between the two in the monograph. A titration with perchloric acid was decided upon for the assay of lumefantrine API. An alternative assay method (not included in the Ph.Int. monograph) was developed, using UV spectrophotometry with an $A_{1cm}^{1\%}$ value of 331.4 determined in methanol. This method was developed in conjunction with the identification test and could be used in cases where a reference standard is not readily available.

The proposed lumefantrine API monograph that had been developed during this study (Appendix B), was adopted for inclusion in the Ph.Int., during the 42nd meeting of the WHO's Expert Committee on Specifications for Pharmaceutical Preparations (July 2008, Geneva). This monograph has ever since served as a quality standard in ensuring the availability of lumefantrine raw materials of acceptable quality.

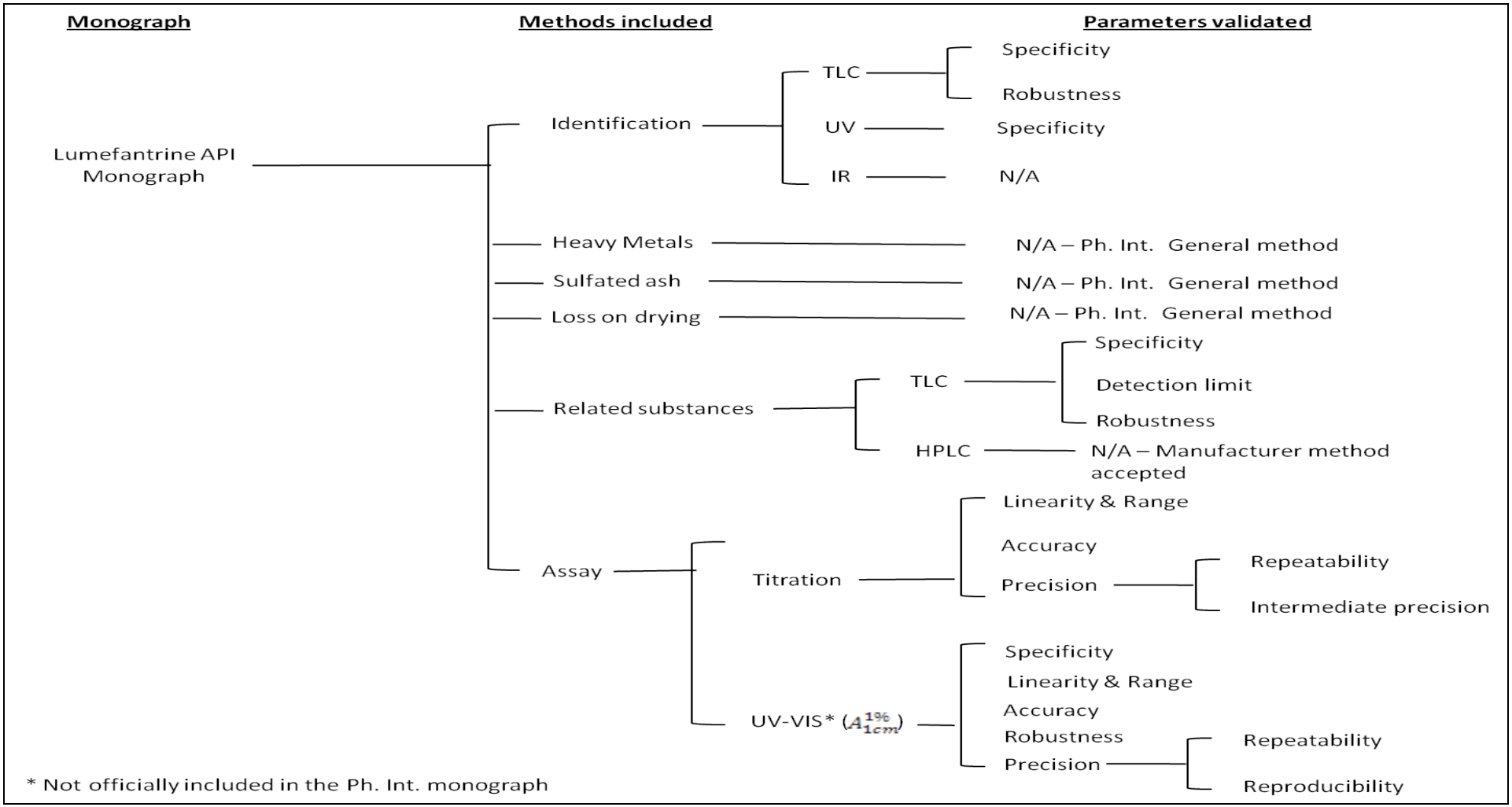


Figure 7.2 Schematic representation of the methods developed during this study and of the parameters validated for the lumefantrine API monograph.

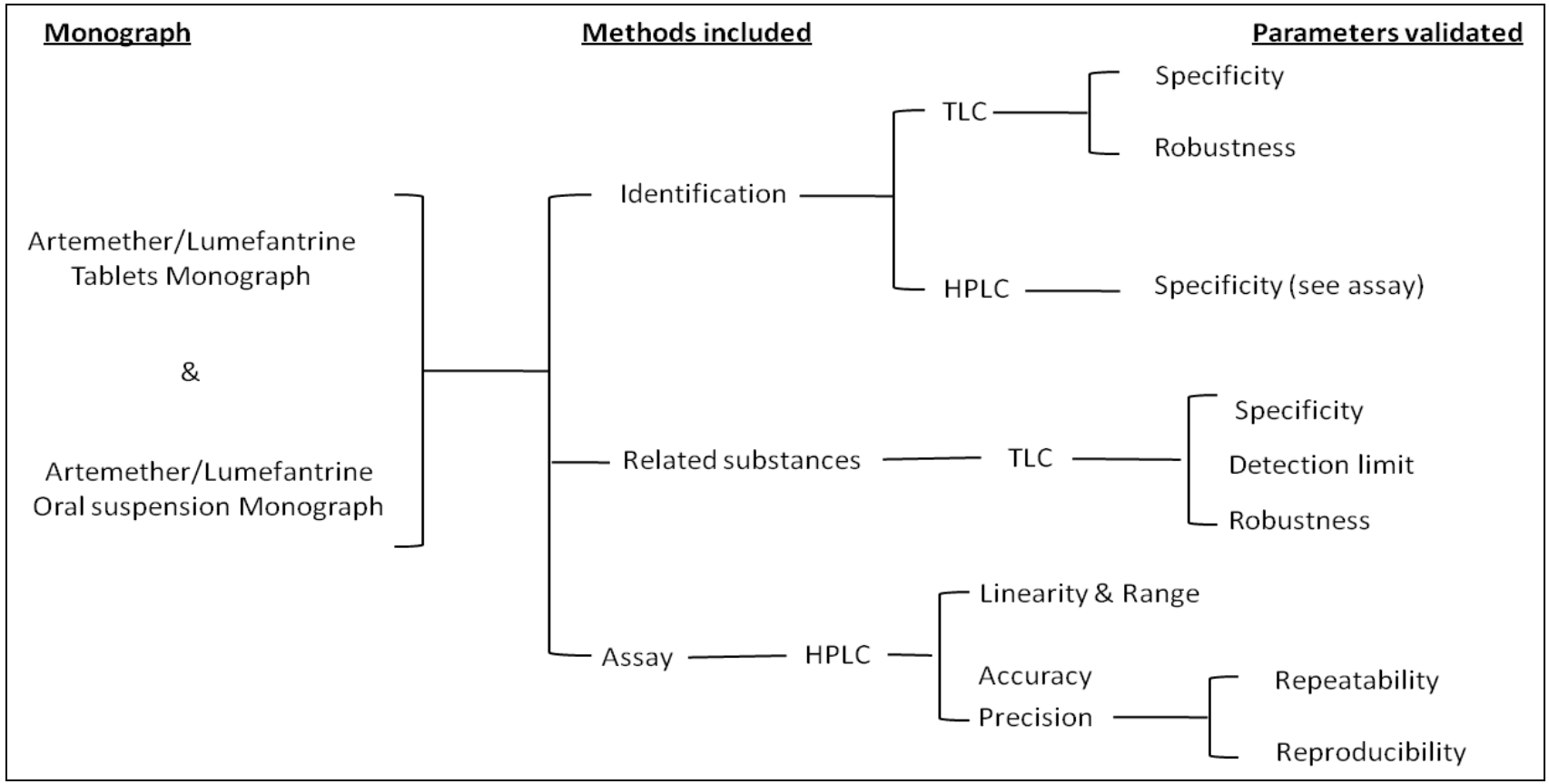


Figure 7.3 Schematic representation of the methods developed during this study and of the parameters validated for the artemether/lumefantrine monographs.

7.3 STAGE 2: Development of monographs for the artemether/lumefantrine dosage forms (tablets and oral suspensions)

In Chapter 5, the methods developed for inclusion in the monographs of the artemether/lumefantrine tablets and powder for oral suspensions, were discussed. For the development of the artemether/lumefantrine oral suspension methods, (powder for suspension) the same methods that had been developed and validated for the tablets, could be used, with only minor adjustments needed in sample preparation. This approach simplified and harmonised the quality control testing of artemether/lumefantrine dosage forms.

Figure 7.3 summarises all the methods developed and lists the parameters validated for these FDC products. For the simultaneous identification of both the APIs, two methods were developed for use in combination, namely a TLC and an HPLC method. As artemether proved the least stable of the two APIs, a TLC limit test was developed for the related substances of artemether. The assay of artemether and lumefantrine could be determined simultaneously, using the HPLC method, thus offering a time and cost effective test method.

The final monographs (Appendix B) were submitted to and adopted for inclusion in the Ph.Int., during the 42nd meeting of the WHO's Expert Committee on Specifications for Pharmaceutical Preparations (July 2008, Geneva). These monographs have ever since served as quality standards to ensure the availability of artemether/lumefantrine products of acceptable quality.

7.4 STAGE 3: Assessment and application of the developed monograph for tablets

The effectiveness of the artemether/lumefantrine tablets monograph was evaluated in Chapter 6 by:

- Evaluating the method transfer (method verification) results of selected methods in the monograph; and
- Utilising the monograph for the testing of commercially available samples.

The results of the method verification tests performed corresponded to those obtained during the validation processes, thus illustrating that the methods were easily transferable between independent QC laboratories.

A quality survey was conducted, in collaboration with the WHO, Geneva, to evaluate the quality of 108 batches of artemether/lumefantrine tablets, as sourced by the WHO in selected Sub-Saharan African countries. The identification, assay and related substances tests in the monographs were utilised for this purpose. The outcomes from this study identified batches of substandard quality and therefore proved that the methods in the monographs were capable of distinguishing between products of acceptable and inferior quality, or even counterfeits.

The results from this study were recently published in the WHO's *Survey of the quality of selected antimalarial medicines circulating in six countries of Sub-Saharan Africa* (WHO, 2011). This manuscript indicated that the monograph would in future continue to serve as a valuable tool in assisting in the detection of counterfeit and substandard antimalarial products.

The conclusion that could be drawn from the outcomes of this study was that the use of the methods in these newly adopted monographs during quality control testing, should indeed result in a reduction in substandard and/or counterfeit products being distributed to the public, thus in reducing the number of malaria related deaths and the escalating occurrence of resistance against inferior antimalarial treatment regimes.

Finally, it is believed that the adoption of the proposed new monographs for inclusion into the Ph.Int. would make a valuable contribution in assisting the WHO in its protection of effective antimalarial treatment regimes and in ultimately helping to improve the quality of lives and communities.

"When you cease to make a contribution, you begin to die." (Eleanor Roosevelt)

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BIBLIOGRAPHY

Aims and Objectives

LINDENBURG, M., KOPP, S. & DRESSMAN, J.B. 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. *European Journal of pharmaceutics and biopharmaceutics*, 58:265-278.

THE INTERNATIONAL PHARMACOPOEIA. 2008. World Health Organization. Available at: http://apps.who.int/phint/en/p/about/ [Date of access: 26 February 2010].

WORLD HEALTH ORGANIZATION. 2006. WHO guidelines for the treatment of malaria. WHO Library Cataloguing-in-Publication, Geneva, Switzerland.

Chapter 1

BJORKMAN, A. 2002. Malaria associated anemia, drug resistance and antimalarial combination therapy. *International Journal of Parasitology,* 32:1637-1643.

BOLAND, P.B. 2001. Drug resistance in malaria. *Malaria epidemiology branch centers for disease control and prevention*, Chamblee. (WHO/CDS/CSR/DRS/2001.4), 27p.

BUABENG, K.O., DUWIEJUA, M., MATOWE, L.K., SMITH, F. & ENLUND, H. 2008. Availability and choice of antimalarials at medicine outlets in Ghana: the question of access to effective medicines for malaria control. *Clinical Pharmacology and Therapeutics*, 84(5):613-619.

CENTRE FOR DISEASE CONTROL (CDC). 2007. Department of health and human services: centers for disease control and prevention: malaria facts: malaria worldwide. Available at: http://www.cdc.gov/malaria/facts.html [Date of access: 23 April 2009].

COHEN-KOHLER, J.C. 2007. The morally uncomfortable global drug gap. *Clinical Pharmacology and Therapeutics*, 82(5):610-614. Available at: http://www.nature.com/clpt/journal/v82/n5/full/6100359a.html [Date of access: 16 June 2009].

DEPARTMENT OF HEALTH. 2006. Malaria manual for community and health workers. *Department of Health, South Africa & World Health Organization.* 50p.

DIRECTORS OF HEALTH PROMOTION AND EDUCATION, MALARIA. 2007. Available at: http://www.dhpe.org/infect/Malaria.html [Date of access: 17 November 2009].

DORSEY, G., STAEDKE, S., CLARK, T.D., NJAMA-MEYA, D., NZARUBARA, B., MAITEKI-SEBUGUZI, C., DOKOMAJILAR, C., KAMYA, M.R. & ROSENTHAL, P.J. 2007. Combination therapy for uncomplicated *falciparum* malaria in Ugandan children. *American Medical Association. JAMA*, 297(20):2210-2219. [www.jama.com at SWETS INFORMATION SERVICES GMBH, 31 July 2007].

HARDMAN, J.G., LIMBIRD, L.E., MOLINOFF, P.B., RUDDON, R.W. & GILMAN, A.G. 1996. Goodman & Gilman's The pharmacological basis of therapeutics. 9th ed. McGraw Hill, International edition. p 1905.

HETZEL, M.W., OBRIST, B., LENGELER, C., MSECHU, J.J., NATHAN, R. DILLIP, A. MAKEMBA, A.M., MSHANA, C., SHCULZE, A. & MSHINDA, H. 2008. Obstacles to prompt and effective malaria treatment lead to low community-coverage in two rural districts of Tanzania. *BMC Public Health*, 8:317. Available at: http://www.biomedcentral.com/1471-2458/8/317 [Date of access: 17 November 2009].

HICKMAN, C.P., ROBERTS, L.S. & LARSON, A. 1997. Integrated principles of zoology. 10th ed. USA: Wm. C. Brown Publishers. 901p.

INTERNATIONAL MEDICAL CORPS (IMC). 2007. Newsroom: malaria fact sheet (updated 27 April 2007). Available at: http://www.imcworldwide.org [Date of access: 19 October 2007].

IMCI INFORMATION PACKAGE. 1999. World Health Organization. Available at: http://whqlibdoc.who.int/hq/1998/WHO_CHS_CAH_98.1B_eng.pdf [Date of access: 17 November 2009].

MILLER, L.H., GOOD, M.F. & MILON, G. 1994. Malaria pathogenesis. *Science*, 264:1878-1883.

MOLYNEUX, D.H., FLOYD, K., BARNISH, G. & FEVRE, E.M. 1999. Transmission, control and drug resistance in malaria: a crucial interaction. *Parasitology Today*, 15(6):238-240.

NEWTON, P.N., GREEN, M.D., FERNANDEZ, F., DAY, N.P.J. & WHITE, N.J. 2006a. Counterfeit anti-infective drugs. Available at: http://infection.thelancet.com, Vol 6 September 2006, 602-613. [Date of access: 16 June 2009].

NEWTON, P.N., McGREADY, R., FERNANDEZ, F., GREEN, M.D., SUNJIO, M., BRUNETON, C., PHANOUVONG, S., MILLET, P., WHITTY, C.J.M., TALISUNA, A.O., PROUX, S., CHRISTOPHEL, E.M., MALENGA, G., SINGHASIVANON, P., BOJANG, K., KAUR, H., PALMER, K., DAY, N.P.J., GREENWOOD, B.M., NOSTEN, F. & WHITE, N.J. 2006b. Manslaughter by fake artesunate in Asia: will Africa be next? PLoS Med 3(6):. doi:10.1371/journal.pmed.0030197. 752-755. [Date of access: 13 June 2006].

OGUNDIPE, S. 2009. Nigeria: NAFDAC seizes 32 million fake anti-malarial drugs. *All africa.com.* Available at: http://allafrica.com/stories/printable/200906020015.html [Date of access: 16 July 2009].

ROLL BACK MALARIA (RBM). 2009. Roll back malaria partnership: 2001-2010: United Nations decade to roll back malaria. Available at: www.rollbackmalaria.org [Date of access: 6 April 2009].

SHAKOOR, O., TAYLOR, R.B. & BEHRENS, R.H. 1997. Assessment of the incidence of substandard drugs in developing countries. *Tropical Medicine and International Health*, 2(9)839-845.

TDR NEWS. 2007. Reducing infant deaths due to malaria. *Special Programe for Research and Training in Tropical Diseases (TDR) sponsored by UNICEF/UNDP/World Bank/WHO.* Feb 2007, no 77, p32.

TDR NEWS. 2008. Eradication is goal: new malaria research initiative launched by RBM partners. *Special Programe for Research and Training in Tropical Diseases (TDR) sponsored by UNICEF/UNDP/World Bank/WHO.* Nov 2008, no 81, p36.

TIERNEY, L.M., McPHEE, S.J. & PAPADAKIS, M.A. 2007. Current medical diagnosis and treatment. 46th ed. USA : The McGraw-Hill Companies. 1931p.

USAID, U.S. Pharmacopoeia. 2007. Drug quality and information program. Available at: http://www.usp.org/worldwide/dqi/index.html [Date of access: 17 November 2009].

UNITED STATES FEDERAL FOOD, DRUG AND COSMETIC ACT (US FDA). 2009a. FDA approves coartem tablets to treat malaria. Available at: http://www.fda.gov/NewsEvents/Newsroom/Pressanouncements/ucm149559.htm News & Events, FDA News Release 8 April 2009. [Date of access: 17 November 2009].

UNITED STATES FEDERAL FOOD, DRUG AND COSMETIC ACT (US FDA). 2009b. Available at: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticAct [Date of access: 16 July 2009].

VALECHA, N., SRIVASTAVA, P., MOHANTY, S.S., MITTRA, P., SHARMA, S.K., TYAGI, P.K., PRADHAN, K., DEV, V., SINGH, R., DASH, A.P. & SHARMA, Y.D. 2009. Therapeutic efficacy of artemether-lumefantrine in uncomplicated *falciparum* malaria in India. *Malaria Journal,* 8:107. Available at: http://www.malariajournal.com/content/8/1/107 [Date of access: 19 May 2009].

WERNSDORFER, W.H. 1991. The development and spread of drug-resistant malaria. *Parasitology Today*, 17(11):297-303.

WORLD HEALTH ORGANIZATION. 2000. Malaria. 20th report of the WHO expert committee. WHO technical report series 929, Geneva. 85p.

WORLD HEALTH ORGANIZATION. 2006. WHO guidelines for the treatment of malaria. WHO Library Cataloguing-in-Publication, Geneva, Switzerland. Available at: http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html. [Date of access: 20 October 2009].

WORLD HEALTH ORGANIZATION. 2009a. WHO: 10 facts on malaria. Available at: http://www.who.int/features/factfiles/malaria/en/index.html, http://www.who.int/mediacentre/factsheets/fs094/en/print.html [Date of access: 7 March 2009].

WORLD HEALTH ORGANIZATION. 2009b. WHO recommended insecticides for indoor residual spraying against malaria vectors. Available at: http://apps.who.int/mmalaria/cmc_upload/0/000/012/604/IRSInsecticides.htm [Date of access: 18 November 2009].

WORLD HEALTH ORGANIZATION. 2009c. Access to medicines. Statement 13 March 2009. Available at: http://www.who.int/mediacentre/news/statements/2009/access-medicines [Date of access: 14 May 2009].

ZIANCE, R.J. 2008. Roles for pharmacy in combating counterfeit drugs, *Journal of the American Pharmacists Association*, 48(4):e71-88.

Chapter 2

BRITISH PHARMACOPOEIA. 2005. Monograph development: guidance to manufacturers. Available at: http://www.bp.pharmacopoeia.co.uk/bp2005/ [Date of access: 7 September 2006].

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH). 2000. Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances (Q6A). 21p + Decision trees.

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH). 2005. Validation of analytical procedures: text and methodology (Q2 (R1)). 17p.

KRISTENSEN, H.G. 2004. Speech by H.G. Kristensen, Chair, European Pharmacopoeia Commission. 40th anniversary of the European Pharmacopoeia, Strasbourg, 15 June 2004. Available at: http://www.coe.int/T/E/Com/Files/Events/2004-05-pharmacopee/disc_kristensen.asp [Date of access: 21 May 2009].

THE INTERNATIONAL PHARMACOPOEIA. 2006. 4th ed. Volume 2. Geneva : WHO. p713-1499.

THE INTERNATIONAL PHARMACOPOEIA. 2008. 4th ed. First Supplement. Geneva : WHO. 365p.

UNITED STATES PHARMACOPOEIAL CONVENTION. 2005. USP 28 **:** NF 23. Rockville, MD. 3187p.

WORLD HEALTH ORGANIZATION. 2005. WHO expert committee on specifications for pharmaceutical preparations. 39th report. Annex 5. Guidelines for registration of fixed-dose combination medicinal products. WHO technical report series 929. Geneva. p95-141.

WORLD HEALTH ORGANIZATION. 2010. The International Pharmacopoeia. Available at: http://www.who.int/medicines/publications/pharmacopoeia/mono-develop.pdf [Date of access: 26 February 2010].

YUWONO, M. & INDRAYANTO, I. 2005. Validation of chromatographic methods of analysis. (*In* Brittain, H.G. *ed.* Profiles of drug substances, excipients, and related methodology). Volume 32. New Jersey : Academic Press. p243-259.

Chapter 3

ABELLI, C., ANDRIOLLO, O., MACHURON, L., VIDEAU, J.Y., VENNAT, B. & POUGET, M.P. 2001. Pharmaceutical equivalence of generic essential drugs. *STP Pharma Praqtiques,* 11(2):102-115.

BYRN, S.R., PFEIFFER, R.R. & STOWELL, J.G. 1999. Solid state chemistry of drugs. 2nd ed. West Lafayette, Indiana : SSCI Inc. 576p.

DAVIDOVICH, M., DIMARCO, J., GOUGOUTAS, J.Z., SCARINGE, R.P., VITEZ, I. & YIN, S. 2005. Detection of polymorphism by powder X-ray diffraction: interference by preferred orientation. 2005 Denver X-ray Conference, Plenary session "X-ray imaging". Sheraton, Colorado Springs, USA. Available at: http://www.dxcicdd.com/05/PDF/M_Davidovich_1.pdf [Date of access: 2 September 2010].

DE, S., RAFEEQ, M., SATHYA-NARAYANA, S. 2006. Polymorphic form I of lumefantrine and processes for its preparation. World Intellectual Property Organization. International publication number: WO 2006/117616 A1. 19p.

DENG, R.X., ZONG, J.X., ALLMENDINGER, T., RIHS, G., PAULUS, K., SUTTER, B., WEBER, W. & WIRZ, P. 2006. The structure of lumefantrine. Available at: http://www.malariaandhealth.com/home/professionalinformation /poster.htm 18p. [Date of access: 24 November 2006].

DJIMDÉ, A. & LEFÈVRE, G. 2009. Understanding the pharmacokinetics of Coartem®. *Malaria Journal,* 8 (Supplement 1):S4 8p. Available at: http://www.malariajournal.com/content/8/S1/Sa [Date of access: 24 March 2010].

EZZET, F., VAN VUGT, M., NOSTEN, F., LOOAREESUWAN, S. & WHITE, N.J. 2000. Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute *falciparum* malaria. *Antimicrobial Agents and Chemotherapy*, 44(3):697-704.

GALICHET, L.Y., MOFFAT, C.A., OSSELTON, M.D. & WIDDOP, B. 2004. Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material. 3rd ed. Volume 2. London : Pharmaceutical Press. 1935p.

GRANT, D.J.W. 1999. Theory and origin of polymorphism. (*In* Brittain, H.G. *ed.* Polymorphism in pharmaceutical solids). New York, Basel : Marcel Dekker. 427p.

HENWOOD, S.Q., DE VILLIERS, M.M., LIEBENBERG, W. & LÖTTER. A.P. 2000. Solubility and dissolution properties of generic rifampicin raw materials. *Drug Development and Industrial Pharmacy*, 26(4):403-408.

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH). 2000. Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances (Q6A). 21p + Decision trees.

LIEBENBERG, W., DEKKER, T.G., LÖTTER. A.P. & DE VILLIERS, M.M. 1998. Identification of the mebendazole polymorphic form present in raw materials and tablets available in South Africa. *Drug Development and Industrial Pharmacy*, 24(5): 485-488.

LIEBENBERG, W., DE VILLIERS, M.M., SWANEPOEL, E., WURSTER, D.E., DEKKER, T.G. & LÖTTER. A.P. 1999. The effect of polymorphism on powder compaction and dissolution properties of chemically equivalent oxytetracycline hydrochloride powders. *Drug Development and Industrial Pharmacy*, 25(9):1027-1033.

O'NEIL, M.J., HECKELMAN, P.E., KOCH, C.B., ROMAN, K.J., KENNY, C.M. & D'ARECCA, M.R. 2006. The Merck Index: an encyclopedia of chemicals, drugs and biologicals. 14th ed. Merck & Co. Inc. : Whitehouse Station, NJ, USA. 10197p.

PECHARSKY, V.K. & ZAVALIJ, P.Y. 2005. Fundamentals of powder diffraction and structural characterization of materials. Springer Science & Business Media, Inc. : New York, USA. 713p.

RANG, H.P., DALE, M.M., RITTER, J.M. & MOORE, P.K. 2003. Pharmacology. 5th ed. London : Churchill Livingstone. 797p.

ROBERTS, S.N.C., WILLIAMS, A.C., GRIMSEY, I.M. & BOOTH, S.W. 2002. Quantitative analysis of mannitol polymorphs. X-ray powder diffractometry: exploring preferred orientation effects. *Journal of Pharmaceutical and Biomedical Analysis,* 28:1149-1159.

RUSH, M.A., BANIECKI, M.L., MAZITSCHEK, R., CORTESE, J.F., WIEGAND, R., CLARDY, J. & WIRTH, D.F. 2009. Colorimetric high-throughput screen for detection of heme crystallization inhibitors. *Antimicrobial Agents and Chemotherapy,* 53(6):2564-2568.

SHAPIRO, T.A. & GOLDBERG, D.E. 2010. Chemotherapy of protozoal infections: malaria (*In* Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed.). http://www.accessmedicine.com/resourceTOC.aspx?resourceID=28 [Date of access: 14 September 2010].

SHEK, F.L.Y., LO, S.M.F., HEYNS, R.K., TSANG, H.W., LAI, L.M. & WILLIAMS, I.D. 2001. Investigation of drug polymorphism and stability by solvothermal crystallization: case of artemisinin and its derivatives [Date of access: 24 March 2010].

SWANEPOEL, E., LIEBENBERG, W., DE VILLIERS, M.M. & DEKKER, T.G. 2000. Dissolution properties of piroxicam powders and capsules as a function of particle size and the agglomeration of powders. *Drug Development and Industrial Pharmacy*, 26(10):1067-1076.

TERBLANCHE, R.J., LIEBENBERG, W., DE VILLIERS, M.M. & LÖTTER. A.P. 2000. Characterisation of zopiclone crystal forms found amongst generic raw materials. *Drug Development and Industrial Pharmacy*, 26(5):531-537.

THE INTERNATIONAL PHARMACOPOEIA. 2008. World Health Organization. Available at: http://apps.who.int/phint/en/p/about/ [Date of access: 26 February 2010].

VIPPAGUNTA, S.R., BRITTAIN, H.G. & GRANT, D.J.W. 2001. Crystalline solids. *Advanced Drug Delivery Reviews,* 48:3-26.

VISERAS, C., SALEM, I.I., GALAN, I.C.G., GALAN, A.C. & GALINDO, A.L. 1995. The effect of recrystallization on the crystal growth, melting point and solubility of ketoconazole. *Thermochimica Acta,* 268:143-151.

XUE, Y., ZHAO, Q. & LUAN, C. 2001. The thermodynamic relations between the melting point and the size of crystals. *Journal of Colloid and Interface Science,* 243:388-390.

YU, L.X., FURNESS, M.S., RAW, A., WOODLAND OUTLAW, K.P., NASHED, N.E., RAMOS, E., MILLER, S.P.F., ADAMS, R.C., FANG, F., PATEL, R.M., HOLCOMBE, F.O. (JNR), CHIU, Y. & HUSSAIN, A.S. 2003. Scientific considerations of pharmaceutical solid and polymorphism in abbreviated new drug applications. *Pharmaceutical Research,* 20(4):531-536.

Chapter 4

CHINESE MONOGRAPH. See Pharmacopoeia of the People's Republic of China.

CHRISTOPHE, A.B. 1971. Valley to peak ratio as a measure for the separation of two chromatographic peaks. *Chromatographia,* 4(10):455-458.

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH). 1999. Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances (Q6A). 32p.

MARONA, H.R.N. & SCHAPOVAL, E.E.S. 2001. Development and validation of a nonaqueous titration with perchloric acid to determine sparfloxacin in tablets. *European Journal of Pharmaceutics and Biopharmaceutics*, 52:227-229.

PHARMACOPOEIA OF THE PEOPLE'S REPUBLIC OF CHINA. 2005. English version, p487. [Chemical Industry Press, Volume II] (Supplied by WHO).

THE INTERNATIONAL PHARMACOPOEIA. 2005. World Health Organization. Available at: http://uqu.edu.sa/files2/tiny_mce/plugins/filemanager/files/4290119/International%20Pharmacopoeia.pdf [Date of access: 7 February 2011].

THE INTERNATIONAL PHARMACOPOEIA. 2008. World Health Organization. Available at: http://www.who.int/medicines/publications/pharmacopoeia/mono-develop.pdf [Date of access: 26 February 2010].

WORLD HEALTH ORGANIZATION. 2009. WHO expert committee on specifications for pharmaceutical preparations. 43rd report. Annex 2. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO technical report series 953. Geneva. 161p.

UNITED STATES PHARMACOPOEIAL CONVENTION. 2010. USP 33**:** NF 28. Available at: http://www.uspnf.com/uspnf/pub/index [Date of access: 23 July 2010].

Chapter 5

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH). 2005. Validation of analytical procedures: text and methodology (Q2 (R1)). 17p.

KAZAKEVICH, Y. & LOBRUTTO, R. 2007. HPLC for pharmaceutical scientists. Wiley : New Jersey. 1104p.

THE INTERNATIONAL PHARMACOPOEIA. 2005. World Health Organization. Available at: http://uqu.edu.sa/files2/tiny_mce/plugins/filemanager/files/4290119/International%20Pharmacopoeia.pdf [Date of access: 7 February 2011].

THE INTERNATIONAL PHARMACOPOEIA. 2008. World Health Organization. Available at: http://www.who.int/medicines/publications/pharmacopoeia/mono-develop.pdf [Date of access: 26 February 2010].

WORLD HEALTH ORGANIZATION. 2006. WHO guidelines for the treatment of malaria. WHO Library Cataloguing-in-Publication, Geneva, Switzerland. Available at: http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html. [Date of access: 20 October 2009].

Chapter 6

ANON 1. 2010. Available at: http://en.wikipedia.org/wiki/File:Skewness_Statistics.svg [Date of access: 9 October 2010].

ANON 2. 2010. Available at: http://ellerbruch.nmu.edu/cs255/jnord/three.gif [Date of access: 9 October 2010].

BOLTON, S. 1997. Pharmaceutical statistics: practical and clinical applications. 3rd ed. Marcel Dekker Inc. : New York. 737p.

EDQM. See European Directorate for the Quality of Medicines and Healthcare.

EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES AND HEALTHCARE. 2005. Guideline on validation of analytical procedures. Available at: http://www.edqm.eu/medias/fichiers/Validation_of_Analytical_Procedures.pdf [Date of access: 8 November 2010].

STEYN, A.G.W., SMIT, C.F., DU TOIT, S.C.H & STRASHEIM, C. 2003. Modern statistics in practice. Van Schaik : Pretoria. 764p.

UNITED STATES PHARMACOPEIAL CONVENTION. 2010. USP 28**:** NF 23 : Rockville, MD. 3187p.

ZIANCE, R.J. 2008. Roles for pharmacy in combating counterfeit drugs. *Journal of the American Pharmacists Association*, 48(4e):71-88.

Chapter 7

UNITED STATES FEDERAL FOOD, DRUG AND COSMETIC ACT (US FDA). 2009. FDA approves coartem tablets to treat malaria. Available at: http://www.fda.gov/NewsEvents/Newsroom/Pressanouncements/ucm149559.htm News & Events, FDA News Release 8 April 2009. [Date of access: 17 November 2009].

WORLD HEALTH ORGANIZATION. 2009. WHO: 10 facts on malaria. Available at: http://www.who.int/features/factfiles/malaria/en/index.html, http://www.who.int/mediacentre/factsheets/fs094/en/print.html [Date of access: 7 March 2009].

WORLD HEALTH ORGANIZATION. 2011. Survey of the quality of selected antimalarials medicines circulating in six countries of sub-Saharan Africa. WHO Library Cataloguing-in-Publication, Geneva, Switzerland.

APPENDIX A

Solvents used for recrystallisation of artemether and lumefantrine

Table 1 Solvents used for recrystallisation of lumefantrine and artemether

Solvent	Manufacturer	Boiling point (°C)	Molecular mass
Ethyl acetate	Saarchem, South Africa	77.00	88.11
Acetone	Saarchem, South Africa	56.50	58.10
Methanol	Merck, Germany	64.65	32.04
Ethanol	Saarchem, South Africa	78.50	46.07
Dichloromethane	Merck, Germany	39.75	84.93
Diethylether	ACE Pty. Ltd., South Africa	35.00	74.12
Iso-propanol	Saarchem, South Africa	82.50	60.10
n-Buntanol	Riedel-de Haën	117.00	74.12
n-Propanol	Saarchem, South Africa	97.20	60.10
Chloroform	Saarchem, South Africa	61.50	119.38
2-Butanol	Riedel-de Haën	98.5-100	74.12
Tetrahydrofuran (THF)	BDH Laboratory Suppliers. England	66.00	72.11
Toluene	Saarchem, South Africa	110.1-111.1	92.14
Acetonitrile (ACN)	BDH Laboratory Suppliers. England	76.00	41.05
Dimethylformamide (DMF)	Merck, Germany	153.00	73.09

APPENDIX B

Monographs accepted in Ph.Int. (2008)

LUMEFANTRINE

ARTEMETHER AND LUMEFANTRINE TABLETS

ARTEMETHER AND LUMEFANTRINE ORAL SUSPENSION

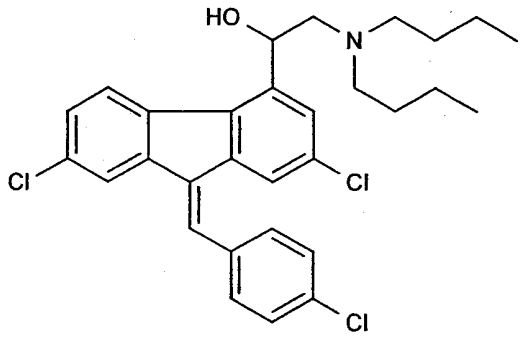
Document QAS/06.186/FINAL
July 2008

World Health Organization

LUMEFANTRINE:
Final text for addition to *The International Pharmacopoeia* (July 2008)

This monograph was adopted at the Forty-second WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2007 for addition to the 4th edition of The International Pharmacopoeia.

LUMEFANTRINUM
LUMEFANTRINE



$C_{30}H_{32}Cl_3NO$

Relative molecular mass. 528.9

Chemical name. 2-Dibutylamino-1-[2, 7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]-ethanol (racemate); CAS Reg. No. 82186-77-4

***[Note from Secretariat:** Name and structure to be checked.]*

Other name. Benflumetol.

Description. A yellow crystalline powder.

Solubility. Practically insoluble in water; soluble in dichloromethane R; slightly soluble in methanol R .

Category. Antimalarial.

Storage. Lumefantrine should be kept in a well-closed container.

Additional information. Lumefantrine melts at 128–132°C.

Requirements

Definition. Lumefantrine contains not less than 98.5% and not more than 101.0% of $C_{30}H_{32}Cl_3NO$, calculated with reference to the dried substance.

Identity test

- Either tests A and B or test C may be applied.

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 40 volumes of light petroleum R1, 6 volumes of ethyl acetate R and 10 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in ethyl acetate R, containing (A) 10 mg of the test substance per ml and (B) 10 mg of lumefantrine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B.

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air and expose to iodine vapour until spots appear. Examine the chromatogram immediately in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B.

B. Dissolve about 20 mg, accurately weighed, in 200 ml of methanol R by sonication for about 15 minutes. Allow the solution to cool to room temperature and dilute fivefold with methanol R. The absorption spectrum (as described under method 1.6) of the diluted solution when observed between 275 and 325 nm, exhibits a maximum at about 302 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 314 and 348.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from lumefantrine RS or with the *reference spectrum* of lumefantrine.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3 and determine the heavy metals content according to Method A; not more than 10 µg/g.

Sulfated ash (2.3). Not more than 1.0 mg/g.

Loss on Drying. Dry for 3 hours at 105°C; it loses not more than 5.0 mg/g.

Related substances

- Either test A or test B may be applied

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (12.5 cm x 4.0 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm)[1].

Use the following conditions for gradient elution:

Mobile phase A: 200 volumes of ion pair reagent, 500 volumes of water R, 250 volumes of acetonitrile R and 50 volumes of 1-propanol R.

Mobile phase B: 200 volumes of ion pair reagent, 100 volumes of water R, 650 volumes of acetonitrile R and 50 volumes of 1-propanol R.

Mobile phase C: 100 volumes of water R, 100 volumes of acetonitrile R and 400 volumes of 1-propanol R.

Prepare the ion pair reagent by dissolving 5.65 g of sodium hexanesulfonate R and 2.75 g of sodium dihydrogen phosphate R in about 900 ml of water R. Adjust the pH to 2.3 using phosphoric acid (~105 g/l) TS, dilute to 1000 ml and filter through a 0.5 µm filter.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Mobile phase C (% v/v)	Comments
0-14	25	75	0	Isocratic
14-19	25 to 0	75 to 100	0	Linear gradient
19-20	0	100 to 80	0 to 20	Linear gradient
20-26	0	80	20	Isocratic
26-27	0	80 to 30	20 to 70	Linear gradient
27-50	0	30	70	Isocratic
50-51	0 to 25	30 to 75	70 to 0	Return to initial composition
51-56	25	75		Re-equilibration

[1] Nucleosil-100 is suitable.

Prepare the following solutions in acetonitrile R. For solution (1) use 0.3 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.3 µg of lumefantrine per ml. For solution (3) dissolve 3 mg of lumefantrine for system suitability RS (containing lumefantrine and impurities A, B and C) in 10 ml.
Operate with a flow rate of 2.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 265 nm.

Inject 20 µl of solution (3). The impurity peaks are eluted at the following relative retention with reference to lumefantrine (retention time about 10 minutes): impurity A about 0.9; impurity B about 4.3 and impurity C about 4.6. The test is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 2.0, where Hp = height above the baseline of the peak due to impurity A and Hv = the height above the baseline of the lowest point of the curve separating this peak from that due to lumefantrine. If necessary adjust the amount of acetonitrile in mobile phase A, or adjust the gradient programme.

Inject alternatively 20 µl each of solutions (1) and (2).

In the chromatogram obtained with solution (1) the area of any individual peak corresponding to either impurity B or impurity C is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%). The area of any other impurity peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak in the chromatogram obtained with solution (2) (0.5 %). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak resulting from the solvent.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 40 volumes of light petroleum R1, 6 volumes of ethyl acetate R and 10 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of solutions (1), (3) and (4). Solution (1) contains 10 mg of the test substance per ml of ethyl acetate R. For solution (2) dilute 1 ml of solution (1) to 100 ml with ethyl acetate R. For solution (3) dilute 3 ml of solution (2) to 10 ml with ethyl acetate R. For solution (4) dilute 2 ml of solution (2) to 20 ml with ethyl acetate R. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

   In the chromatogram obtained with solution (1) any spot, other than the principal spot, is not more intense than that in the chromatogram obtained with solution (3) (0.3%) and not more than two such spots are more intense than that in the chromatogram obtained with solution (4) (0.1%).

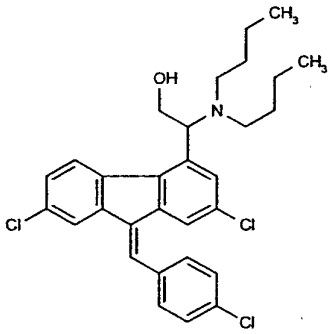
Assay

Dissolve about 0.45 g, accurately weighed, in 50 ml of glacial acetic acid R1 by stirring for about 15 minutes, and titrate with perchloric acid (0.1 mol/l) VS, determine the end-point potentiometrically as described under 2.6 Non aqueous titration, Method A. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 52.89 mg of $C_{30}H_{32}Cl_3NO$.

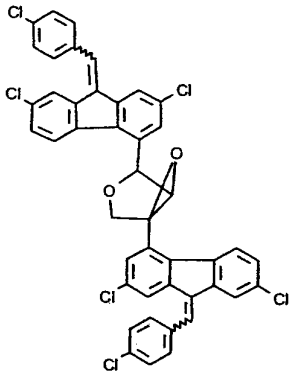
Impurities

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.

A. 529.0 $C_{30}H_{32}Cl_3NO$



B. and C. 797.4 $C_{44}H_{24}Cl_6O_2$



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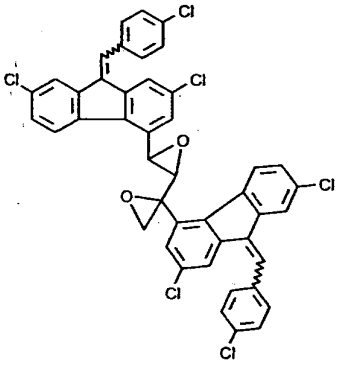


Document QAS/07.192/FINAL
July 2008

World Health Organization

ARTEMETHER AND LUMEFANTRINE TABLETS:

Final text for addition to *The International Pharmacopoeia* (July 2008)

This monograph was adopted at the Forty-second WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2007 for addition to the 4th edition of The International Pharmacopoeia.

Category. Antimalarial.

Storage. Artemether and Lumefantrine tablets should be kept in a well-closed container, protected from light.

Additional information. Strength in the current WHO Model list of essential medicines: 20 mg Artemether and 120 mg Lumefantrine.
Strength in the current WHO Model list of essential medicines for children: 20 mg Artemether and 120 mg Lumefantrine.

Requirements

Comply with the monograph for "Tablets".

Definition. Artemether and Lumefantrine tablets contain Artemether and Lumefantrine. They contain not less than 90.0% and not more than 110.0% of the amounts of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) stated on the label.

Identity tests

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 40 volumes of light petroleum R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions in acetone R. For solution (A) shake a quantity of the powdered tablets containing about 10 mg of Artemether (about 60 mg of Lumefantrine) for 5 minutes with 10 ml, filter, and use the clear filtrate. For solution (B) use 1 mg of artemether RS and 6 mg of lumefantrine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air.

(i) Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B (identifying lumefantrine).

(ii) Spray the plate with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B (identifying artemether; a faint spot due to lumefantrine may also be visible).

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Spray with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C, allow it to cool and expose to iodine vapours for 20 minutes. Examine the chromatogram immediately in daylight.

The principal spots obtained with solution A correspond in position, appearance, and intensity to those obtained with solution B.

B. See the test described below under Assay. The retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

Artemether-related substances. Protect samples from light, also during chromatography.

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 40 volumes of light petroleum R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R as the mobile phase.

Prepare the following solutions in the solvent consisting of 1 volume of water R and 1 volume of acetonitrile R. For solution (1), weigh and powder 20 tablets. To a quantity of the powder containing 100 mg of Artemether add 20 ml of the solvent, sonicate for 15 minutes and centrifuge. Filter a portion of the supernatant through a 0.45-µm filter, discarding the first few ml of the filtrate. For solution (2) dissolve 5 mg of each of artemether RS, artenimol RS and α-artemether RS in 50 ml of the solvent. For solution (3) dilute 2.0 ml of solution (2) to 20 ml with the solvent. For solution (4) dilute 3.0 ml of solution (2) to 20 ml with the solvent. For solution (5) dilute 5.0 ml of solution (2) to 20 ml with the solvent. For solution (6) dilute 1.0 ml of solution (2) to 2 ml with the solvent. For solution (7) dilute 3.0 ml of solution (2) to 4 ml with the solvent.

Apply separately to the plate 20 µl of each of solutions (1), (3), (4), (5), (6) and (7). After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in vanillin/sulfuric acid TS2. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight.

Artemether and related substances have the following R_f values: impurity A about 0.25; impurity B (artenimol) about 0.3; impurity C about 0.35; impurity D (α-artemether) about 0.4; artemether about

0.55. The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

In the chromatogram obtained with solution (1):

- any spot corresponding to impurity A is not more intense than the principal spot in the chromatogram obtained with solution (7) (1.5%),
- any spot corresponding to impurity B is not more intense than the spot due to artenimol in the chromatogram obtained with solution (6) (1.0%),
- any spot corresponding to impurity C is not more intense than the principal spot in the chromatogram obtained with solution (5) (0.5%),
- any spot corresponding to impurity D is not more intense than the spot due to α-artemether in the chromatogram obtained with solution (4) (0.3%),
- any other spot is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot remaining at the point of application.

Assay. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 3.9 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm)[1].

Use the following conditions for gradient elution:

Mobile phase A: 700 volumes of ion pair reagent and 300 volumes of acetonitrile R.

Mobile phase B: 300 volumes of ion pair reagent and 700 volumes of acetonitrile R.

Prepare the ion pair reagent by dissolving 5.65 g of sodium hexanesulfonate R and 2.75 g of sodium dihydrogen phosphate R in about 900 ml of water R. Adjust the pH to 2.3 using phosphoric acid (~105 g/l) TS, dilute to 1000 ml and filter through a 0.45-µm filter.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-28	60	40	Isocratic
28-29	60 to 0	40 to 100	Linear gradient
29-45	0	100	Isocratic
45-46	0 to 60	100 to 40	Return to initial composition
46-55	60	40	Isocratic re-equilibration

Prepare the following solutions in the solvent which is obtained by mixing 200 ml of ion pair reagent, 60 ml of water R and 200 ml of 1-propanol R and diluting to 1000 ml with acetonitrile R. Prepare and keep both solutions at a temperature not below 20°C. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powder containing about 20 mg of Artemether (about 120 mg of Lumefantrine), accurately weighed, to a 100-ml volumetric flask. Add approximately 85 ml of the solvent, sonicate for 20 minutes, allow to cool to room temperature and dilute to volume with the solvent. Filter through a 0.45 µm-filter, discarding the first few ml of the filtrate. For solution (2), accurately weigh 20 mg of artemether RS and 120 mg of lumefantrine RS in a 100 ml

[1] Symmetry is suitable.

volumetric flask. Add approximately 85 ml of solvent, sonicate until dissolved, allow to cool to room temperature and dilute to volume.

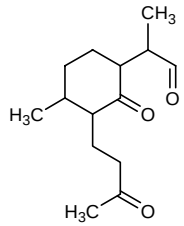
Operate with a flow rate of 1.3 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm for the first 28 minutes and then switch to about 380 nm.

Inject alternately 20 µl each of solutions (1) and (2). (The peak for artemether is eluted at a retention time of approximately 19 minutes, and that for lumefantrine at a retention time of approximately 34 minutes.)

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) in the tablets.

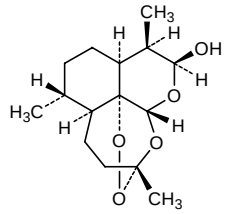
Impurities The impurities limited by the requirements of this monograph include

A.



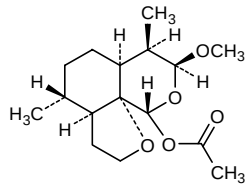
2-[4-methyl-2-oxo-3-(3-oxobutyl)cyclohexyl]propanal

B.



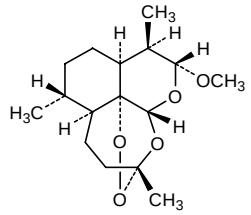
(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol
(artenimol, dihydroartemisinin)

C.



(3a*S*,4*R*,6a*S*,7*R*,8*S*,10*R*,10a*R*)-8-methoxy-4,7-dimethyloctahydro-2*H*-furo[3,2-*i*][2]benzopyran-10-yl acetate

D.



(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-10-methoxy-3,6,9-trimethyldecahydro-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepine
(α-artemether)

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Document QAS/07.217/FINAL
November 2008

World Health Organization

ARTEMETHER AND LUMEFANTRINE ORAL SUSPENSION: Final text for addition to *The International Pharmacopoeia* (November 2008)

Category. Antimalarial.

Storage. Artemether and lumefantrine oral suspension should be stored in a tightly closed container at the temperature stated on the label and used within the period stated on the label.

The powder should be kept in a well-closed container, protected from light.

Additional information. Strength usually available: When the oral suspension is prepared as stated on the label, 15 mg Artemether and 90 mg of Lumefantine per 5 ml (3 mg Artemether and 18mg Lumefantrine per ml).

Usually supplied as bottles of powder containing 180 mg of Artemether with 1080 mg of Lumefantrine for preparation of 60 ml of oral suspension and 360 mg of Artemether with 2160 mg of Lumefantrine for preparation of 120 ml of oral suspension.

Requirements

Complies with the monograph for "Liquid Preparations for Oral Use"; the powder complies with the section of the monograph entitled "Powders for oral solutions, oral suspensions and oral drops" and with the requirements below.

Definition. Artemether and lumefantrine oral suspension is a suspension of Artemether and Lumefantrine in a suitable vehicle; it may be flavoured. It is prepared by suspending the powder in the specified volume of the liquid stated on the label just before issue for use.

The powder contains not less than 90.0% and not more than 110.0% of the amounts of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) stated on the label.

Identity tests

A Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 40 volumes of light petroleum R1, 10

volumes of ethyl acetate R and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions in acetone R. For solution (A) shake a quantity of the powder containing about 10 mg of Artemether for 5 minutes with 10 ml, filter, and use the clear filtrate. For solution (B) use 1 mg of artemether RS and a proportional quantity (according to the ratio in the powder) of lumefantrine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air.

(i) Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B (identifying lumefantrine).

(ii) Spray the plate with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B (identifying artemether; a faint spot due to lumefantrine may also be visible).

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Spray with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C, allow it to cool and expose to iodine vapours for 20 minutes. Examine the chromatogram immediately in daylight.

The principal spots obtained with solution A corresponds in position, appearance, and intensity to those obtained with solution B.

B. See the test described below under Assay. The retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

Artemether-related substances

Protect samples from light, also during chromatography.

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 40 volumes of light petroleum R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R as the mobile phase.

Prepare the following solutions in the solvent consisting of equal volumes of water R and acetonitrile R. For solution (1), shake a quantity of the powder containing 100 mg of Artemether with a mixture of 100 ml water and 4 ml of sodium hydroxide (~40 g/l) TS. Immediately extract with four 15-ml quantities of dichloromethane R and evaporate the combined extracts to dryness. Sonicate the residue with 20 ml of the solvent for 15 minutes, centrifuge and use the clear supernatant. For solution (2) dissolve 5 mg of each of artemether RS, dihydroartemisinin (artenimol RS) and α-artemether RS in 50 ml of the solvent. For solution (3) dilute 2.0 ml of solution (2) to 20 ml with the solvent. For solution (4) dilute 3.0 ml of solution (2) to 20 ml with the solvent. For

solution (5) dilute 5.0 ml of solution (2) to 20 ml with the solvent. For solution (6) dilute 1.0 ml of solution (2) to 2 ml with the solvent. For solution (7) dilute 3.0 ml of solution (2) to 4 ml with the solvent.

Apply separately to the plate 20 µl of each of solutions (1), (3), (4), (5), (6) and (7). After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in vanillin/sulfuric acid TS2. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight.

Artemether and related substances have the following R_f values: impurity A about 0.25; impurity B (artenimol) about 0.3; impurity C about 0.35; impurity D (α-artemether) about 0.4; artemether about 0.55. The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

In the chromatogram obtained with solution (1):

- any spot corresponding to impurity A is not more intense than the principal spot in the chromatogram obtained with solution (7) (1.5%),
- any spot corresponding to impurity B is not more intense than the spot due to artenimol in the chromatogram obtained with solution (6) (1.0%),
- any spot corresponding to impurity C is not more intense than the principal spot in the chromatogram obtained with solution (5) (0.5%),
- any spot corresponding to impurity D is not more intense than the spot due to α-artemether in the chromatogram obtained with solution (4) (0.3%),
- any other spot is not more intense than the principal spot in the chromatogram obtained with solution (3) (0.2%). Disregard any spot remaining at the point of application.

Assay. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 3.9 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm)[1].

Use the following conditions for gradient elution:

Mobile phase A: 700 volumes of ion pair reagent and 300 volumes of acetonitrile R.

Mobile phase B: 300 volumes of ion pair reagent and 700 volumes of acetonitrile R.

Prepare the ion pair reagent by dissolving 5.65 g of sodium hexanesulfonate R and 2.75 g of sodium dihydrogen phosphate R in about 900 ml of water R. Adjust the pH to 2.3 using phosphoric acid (~105 g/l) TS, dilute to 1000 ml and filter through a 0.45 µm filter.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-28	60	40	Isocratic
28-29	60 to 0	40 to 100	Linear gradient

[1] Symmetry is suitable.

29-45	0	100	Isocratic
45-46	0 to 60	100 to 40	Return to initial composition
46-55	60	40	Isocratic re-equilibration

Prepare the following solutions in the solvent which is obtained by mixing 200 ml of ion pair reagent, 60 ml of water R and 200 ml of 1-propanol R and diluting to 1000 ml with acetonitrile R. Prepare and keep both solutions at a temperature not below 20°C. For solution (1), weigh the contents of a container. To a quantity of the powder containing about 20 mg of Artemether, accurately weighed, add 100 ml of the solvent, sonicate for 20 minutes and allow to cool to room temperature. Filter through a 0.45-µm filter, discarding the first few ml of the filtrate. For solution (2), accurately weigh 20 mg artemether RS and a proportional quantity (according to the ratio in the powder) of lumefantrine RS in a 100 ml volumetric flask. Add approximately 85 ml of solvent, sonicate until dissolved, allow to cool to room temperature and dilute to volume.

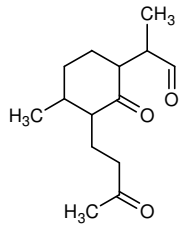
Operate with a flow rate of 1.3 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm for the first 28 minutes and then switch to about 380 nm.

Inject alternately 20 µl each of solutions (1) and (2). (The peak for artemether is eluted at a retention time of approximately 19 minutes, and that for lumefantrine at a retention time of approximately 34 minutes.)

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) in the powder .

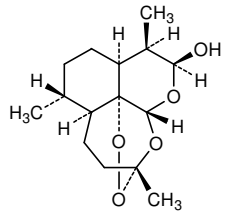
Impurities The impurities limited by the requirements of this monograph include

A.



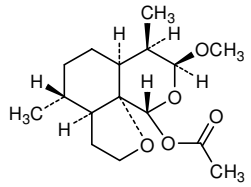
2-[4-methyl-2-oxo-3-(3-oxobutyl)cyclohexyl]propanal

B.



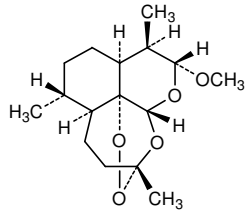
(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-3,6,9-trimethyldecahydro-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol
(artenimol, dihydroartemisinin)

C.



(3a*S*,4*R*,6a*S*,7*R*,8*S*,10*R*,10a*R*)-8-methoxy-4,7-dimethyloctahydro-2*H*-furo[3,2-*i*][2]benzopyran-10-yl acetate

D.



(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-10-methoxy-3,6,9-trimethyldecahydro-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepine
(α-artemether)

***

APPENDIX C

Validation of a thin layer chromatographic method for the identification of lumefantrine

PART A: OBJECTIVE

The objective is to validate the thin layer chromatographic method for the identification (semi-quantitive) of lumefantrine active pharmaceutical ingredient. The evaluation and validation of this method will be done according to the ICH Guidelines on Validation of Analytical Procedures Q2(R1) 2005:3. Specificity and robustness will be investigated.

PART B: IDENTIFICATION TEST PROCEDURES

B.1 ORIGIN OF METHOD

Method for artemether/lumefantrine 20/120 mg tablets from manufacturer supplied by the WHO.

B.2 PRINCIPLE OF METHOD

The identification of the lumefantrine API should inimitably be confirmed by means of thin layer chromatography.

B.3 EQUIPMENT

- TLC chamber
- Aluminium TLC plate coated with silica gel R5[1] (Macherey-Nagel, Alugram® SIL G)
- Glass TLC plate coated with silica gel R5[1] (Macherey-Nagel, DC-Fertigplatten® SIL G-25)
- Aluminium TLC plate coated with silica gel R6[2] (Macherey-Nagel, Alugram® SIL G/UV_{254})
- Glass TLC plate coated with silica gel R6[2] (Macherey-Nagel, DC-Fertigplatten® SIL G-25/UV_{254})
- UV source with wavelength 254 nm
- Volumetric glassware (A grade, class 1)

B.4 SAMPLES, REAGENTS, SOLVENTS AND SOLUTIONS

- Lumefantrine primary reference standard (RS), provided by the manufacturer (Table 1)
- Lumefantrine test samples (Table 1)
- Lumefantrine related substance A, provided by the manufacturer (Table 1)

[1] Silica gel 60, a white, homogeneous powder. Average pore size = 6 nm (Ph.Int., 2008)
[2] Silica gel 60 (UV 254), a white, homogeneous powder. Average pore size = 6 nm.
Composition: Silica gel (average particle size = 15 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg) (Ph.Int., 2008).

- Lumefantrine related substances B and C (mixture of isomers), provided by the manufacturer (Table 1)
- Light petroleum ether R1 (boiling point: 40ºC - 60ºC) (Merck, AR grade)
- Ethyl acetate R[3] (Merck, AR grade)
- Glacial acetic acid R (Merck, AR grade)
- Mobile phase: Prepare a mixture of 40 volumes light petroleum ether R1, 6 volumes of ethyl acetate R and 10 volumes of glacial acetic acid R.
- Iodine pellets (Merck, AR grade)

Table 1 Lumefantrine reference standards and test samples used for validation of TLC identification method

Description	Batch number	Manufacturing Company
Primary standard	C0189	Novartis
Lumefantrine test sample 1	070701	Tianjin Hi-tesion Bio & Chem Co., Ltd.
Lumefantrine test sample 2	090306	IFF, Iffect Chemphar (HK) Company Limited
Lumefantrine related substance A	802CB	Novartis
Lumefantrine related substance mixture B and C	6978E	Novartis

B.5 STANDARD SOLUTION

Dissolve 20 mg of lumefantrine RS in 2 ml ethyl acetate R.

B.6 TEST SOLUTIONS

For test solution (1), dissolve 20 mg lumefantrine test sample in 2 ml ethyl acetate R. For test solution (2) dissolve 5 mg lumefantrine related substance A in 1 ml ethyl acetate and for test solution (3) 5 mg of the related substance mixture B and C in 1 ml ethyl acetate R. These test solutions were used in the validation only and is not part of the related substances test.

B.7 MEASUREMENT PROCEDURE

To a silica gel R6 plate separately apply 10 µl of each of the standard solution and the test solutions (1) and (2). After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 10-15 cm in a pre-saturated chromatographic chamber. After removing the TLC plate from the chromatographic chamber, allow it to dry exhaustively in air or

[3] The notation "R" (R1, R2, etc.) is used to define the grade of a reagent, including a solvent, in the Ph.Int. (2008). Other notions used in the Ph.Int. (2008) are for reference substances (RS), test solutions (TS) and volumetric solutions (VS).

in a current of cool air. Examine the TLC plate under UV radiation at 254 nm. Expose the TLC plate to iodine vapour until spots appear. Examine the TLC plate immediately in daylight.

B.8 INTERPRETATION OF RESULTS

Examine the plates visually. Note the position, colour, size and intensity of the spots and calculate the Rf-values.

PART C: VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

C.1 SPECIFICITY

Prepare the standard solution and test solutions (1), (2) and (3) as described in B.5 and B.6. Follow the measurement procedure as described in B.7.

Acceptance criteria:

The principle spot obtained with test solution (1) should correspond to that of the standard solution with respect to appearance, intensity and position (Rf). The spots obtained with test solutions (2) and (3) should not interfere with that obtained with test solution (1) and/or the standard solution. Spots should be visible under UV radiation at 254 nm. After being exposed to iodine vapour, all spots should be visible in daylight.

C.2 ROBUSTNESS

- Allow the test solution (1) (B.6) and standard solution (B.5) as used for specificity testing to stand on the shelf for 24 hours (to determine stability of the test solutions).
- Follow the measurement procedure (B.7), apply the above aged solutions and freshly prepared standard solution (B.5) and test solution (1) (B.6) to both a glass and aluminium silica gel R6 TLC plates.

Acceptance criteria:

- The principle spots obtained with the freshly prepared solution and aged solutions should be visually inspected and compared with regards to appearance, intensity and position (Rf) to determine the stability of standard and test solutions over a period of 24 hours, using both detection methods. No differences in the appearance, intensity and position (Rf) should be detected between the freshly prepared and the aged samples.
- The spots on the aluminium and on the glass plates should separately be inspected and compared, using both detection methods. The aluminium plate and glass plate should each produce results where the spots of the reference standard and the test sample correspond to each other with regards to appearance, intensity and position. The Rf values for the principle spots on the aluminium and glass plates may differ.

PART D: SUMMARY AND DISCUSSION OF VALIDATION RESULTS

Table 2 Summary of validation results for TLC identification method

Parameter	Acceptance criteria	Results
Specificity	The principle spot obtained with test solution (1), which is a solution of the test substance, should correspond to that of the standard solution with regards to appearance, intensity and position. The spots obtained with test solutions (2) and (3), which are solutions of known related substances, should not interfere with that obtained with test solution (1) and/or the standard solution. Spots should be visible under UV radiation at 254 nm. After being exposed to iodine vapour, all spots should be visible in daylight.	The principle spot obtained with test solution (1) corresponded to that of the standard solution with regards to appearance, intensity and position. The spots obtained with test solutions (2) and (3) did not interfere with the spot obtained with test solution (1) or the standard solution. Spots were visible under UV radiation at 254 nm (Figure 1). After being exposed to iodine vapour, all spots were visible in daylight.
Robustness A: Stability of standard and test solutions after standing for 24 hours	The principle spots obtained with test solution (1) and the standard solution after standing for 24 hours on the bench should be comparable to that of freshly prepared solutions, with respect to appearance, intensity and position.	The results obtained on solutions that stood for 24 hours at ambient conditions were comparable to the results of freshly prepared solutions.
B: Use of glass and aluminium plates	Aluminium plate and glass plate should each produce results where the spots of the reference standard and the test sample correspond to each other with regards to appearance, intensity and position. The Rf values of the aluminium and glass plates may differ.	Both the aluminium and glass TLC plates produced results acceptable for identification according to the acceptance criteria (Figure 2).

D.1 SPECIFICITY

A glass plate was used for the specificity test. As illustrated in Figure 1 the principle spot obtained with test solution (1) corresponded to that of the standard solution with regards to appearance, intensity and position (see Rf values in Table 3). The spots obtained with test solutions (2) and (3) did not interfere with that obtained with test solution (1) or the standard solution. Spots were visible under UV radiation at 254 nm. After being exposed to iodine vapour, all spots were visible in daylight.

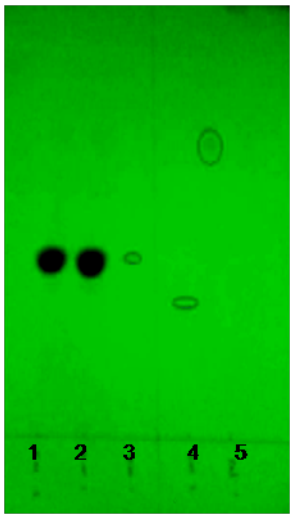


Figure 1 TLC for lumefantrine specificity test under UV light at 254 nm. Spots from left to right: 1 = standard solution; 2 = test solution (1); 3 = test solution (1) diluted (0.1%[4]: of test solution (1) and standard solution); 4 = test solution (2) (related substance A) and 5 = test solution (3) (related substances B and C).

Table 3 Rf values calculated for lumefantrine and related substances (glass plate)

	Lumefantrine sample		**Lumefantrine standard**		**Lumefantrine related substances**	
	mm	Rf	mm	Rf	mm	Rf
Mobile phase	147		147		147	
Principle spot	48	0.33	48	0.33		
Related substance A	-	-	-	-	37	0.25
Related substance B	-	-	-	-	98	0.67
Related substance C	-	-	-	-	100	0.68

[4] Plate also used for related substances test, thus 0.1% (related substances limit) dilution used.

D.2 ROBUSTNESS

For the robustness test, both aluminium and glass plates were used. One standard solution and one test solution (1) for each of the two samples were used. The data in Table 4 show little variation between the spots of the standard and test solutions on the aluminium plate. Similarly, little variation exists between the spots of the standard and test solutions on the glass plate.

The aluminium plate (Rf≈ 0. 18) and glass plate (Rf≈ 0. 30) showed a significant difference in the Rf value of the lumefantrine spot. However, this is not considered important – being not part of the acceptance criteria – since the test is always conducted using one single plate, be it an aluminium plate or a glass plate.

Table 4 Comparison of the Rf values calculated for glass and aluminium plates

	Glass plate		**Aluminium plate**	
	mm	Rf	mm	Rf
Solvent	135		140	
Sample 1	42	0.31	25	0.18
Sample 2	40	0.30	26	0.19
Standard	40	0.30	25	0.18

As seen in Figure 2 there was no significant difference between the freshly prepared sample and the aged sample with respect to appearance, intensity and position. The test and standard solutions should be used within 24 hours after preparation. No comment with respect to the stability of these solutions is needed in the Ph.Int. monograph of lumefantrine API, since the period of 24 hours is sufficiently long for conducting the test.

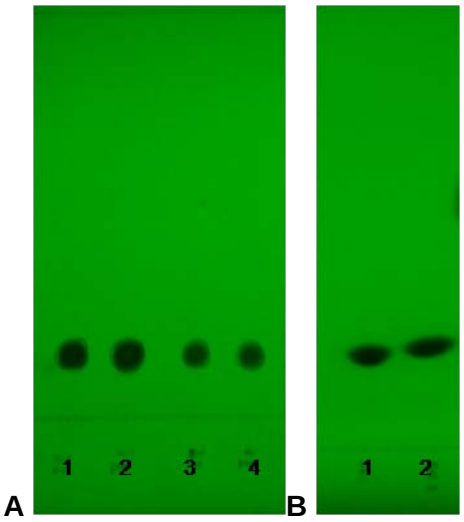


Figure 2 Identification under UV light at 254 nm: Plate A (aluminium) spot 1 = 10 µl test solution (1), spot 2 = 10 µl test solution (1) after 24 hours, spot 3 = 5 µl test solution (1), spot 4 = 5 µl test solution (1) after 24 hours; Plate B (glass plate) spot 1 = 10 µl test solution (1), spot 2 = 10 µl test solution (1) after 24 hours.

PART E: CONCLUSION

A TLC method for the identification of lumefantrine API has been developed and validated (summary of validation results Table 2). According to the Ph.Int. general requirements followed, two methods were developed to visualise the lumefantrine spot. If the test laboratory is equipped with a UV (254 nm) lamp for TLC, this would be the visualisation method of choice; otherwise exposure to iodine vapour could be used. The latter is regarded as a fast, uncomplicated, effective and inexpensive visualisation technique.

This method proved to be specific and robust with respect to either aluminium or glass TLC plates. The known related substances did not interfere in the test. The test and sample solutions were stable for at least 24 hours at ambient conditions.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 4.

APPENDIX D

Validation of a UV assay method and determination of for lumefantrine

PART A: OBJECTIVE

The objective is to determine the (specific absorbance) value for lumefantrine in methanol R, for the assay of lumefantrine raw material. The evaluation and validation of this method will be done according to the ICH Guidelines on Validation of Analytical Procedures Q2(R1) (2005:3). Accuracy, precision (repeatability, intermediate precision), specificity, linearity, range and robustness will be investigated.

PART B: SPECTROPHOTOMETRIC TEST PROCEDURES

B.1 ORIGIN OF METHOD

Self-developed.

B.2 PRINCIPLE OF METHOD

The UV-VIS method for the determination of lumefantrine assay, in methanol R, in terms of . The maximum absorbance of lumefantrine at about 302 nm and spectrum (200 – 400 nm) to be established.

B.3 EQUIPMENT

- Hewlett Packard (8453 UV-VIS) spectrophotometer equipped with a deuterium lamp and quartz cells with 10 mm pathway (Agilent, Germany).
- Beckman (DU650i) spectrophotometer equipped with a deuterium lamp and quartz cells with 10 mm pathway (Beckman Coulter, USA).
- Shimadzu UV-2450 spectrophotometer equipped with halogen and deuterium lamps and quartz cells with 10 mm pathway (Shimadzu, Japan).
- Volumetric glassware (A grade, class 1).
- Millipore filters: 0.45 µm hydrophilic PVDF membrane (Microsep, South Africa).

B.4 REAGENTS AND SOLVENTS

- Lumefantrine primary reference standard (RS), provided by the manufacturer (Table 1)
- Lumefantrine test samples (Table 1)
- Methanol R (Merck, AR grade)

Table 1 Lumefantrine reference standard and test sample used for validation of UV-VIS identification and assay method

Description	Batch number	Manufacturing Company
Primary standard	C0189	Novartis
Lumefantrine test sample	090306	IFF, Iffect Chemphar (HK) Company Limited

B.5 STANDARD SOLUTION

Accurately weigh about 20 mg of lumefantrine RS and transfer quantitatively into a 200 ml volumetric flask. Add about 100 ml methanol R and sonicate for 20 minutes to dissolve. Allow to cool to room temperature, fill to volume with methanol R and mix, the [target concentration = 0.1 mg/ml[1]]. Further dilute 10 ml of this solution to 50 ml with methanol R [target concentration of 0.02 mg/ml[1]]. Filter through a 0.45 µm filter, discard the first few milliliter and use the filtrate.

B.6 TEST SOLUTION FOR LUMEFANTRINE

Accurately weigh 20 mg of lumefantrine test sample and transfer quantitatively into a 200 ml volumetric flask. Add about 100 ml methanol R and sonicate for 20 minutes to dissolve. Allow to cool to room temperature, fill to volume with methanol R and mix; the [target concentration of 0.1 mg/ml[1]]. Further dilute 10 ml of this solution to 50 ml with methanol R [target concentration of 0.02 mg/ml[1]]. Filter through a 0.45 µm filter, discard the first few milliliter and use the filtrate.

B.7 MEASUREMENT PROCEDURE

Determine the absorbance spectra in the range 200 – 400 nm, and identify the wavelength of maximum absorption of the test and standard solutions from these spectra, using methanol R (solvent) to blank the instrument before use. Determine the absorption at the specified wavelength of maximum absorption for the test and sample solutions to be used in the validation (experimentally determined as 302 nm, see D.1).

[1] For target concentrations 0.1 mg/ml use a 2 mm cell. For target concentrations of 0.02 mg/ml use a 10 mm cell.

B.8 CALCULATION OF

The results from lumefantrine RS is used for the calculation

$= A_T / [C]$

$$[C] = \frac{\text{St mass x 100 x P}}{\text{DF x 1000}}$$

A_T = Absorbance of standard solution

St mass = Mass of standard (RS) weighed in mg

DF = Dilution factor of standard solution
(for a 2 mm cell = 200; for a 10 mm cell = 1000)

P = Potency of RS expressed as a fraction of 100

PART C: VALIDATION PARAMETERS, TEST PROCEDURES AND ACCEPTANCE CRITERIA

The ICH guideline Q2(R1) (2005:7) will serve as reference for the validation of this analytical method. The acceptance criteria for the specific validation parameters will be defined in the following sections.

C.1 SPECIFICITY

Record the spectra of the test solution and standard solution (with the solvent as blank) in the region 200 – 400 nm.

Acceptance criterion:

The spectrum of the test solution must conform to that of the standard solution with the solvent used as blank (to ensure no interference of the solvent in the spectra of the standard or test solutions).

C.2 LINEARITY AND RANGE

Linearity and range will be evaluated across a specific range of the analytical procedure.

- Prepare a standard stock solution with concentration of 0.1 mg/ml following the method as described in section B.5.
- Carry out a series of dilutions using the standard stock solution, to obtain solutions in the range of 60%, 80%, 90%, 100%, 110% and 120% of the target concentration of 0.02 mg/ml.

- Measure the absorbance of these solutions in a 10 mm cell at the wavelength of maximum absorbance (302 nm).
- Conduct a least squares linear regression analysis and calculate the correlation coefficient.

Acceptance criterion:

The method should be linear over a range of 60 – 120% with the correlation coefficient $R^2 \geq 0.99$.

C.3 ACCURACY

The accuracy will be assessed using a minimum of six individual standard solutions with concentrations of 0.02 mg/ml of lumefantrine.

- Prepare six standard solutions following the method as described in section B.5, obtaining 100% of the target concentration.
- Measure the absorbance of these solutions at 302 nm as described in B.7.
- Use the equation for the linear regression graph obtained for linearity and range testing (D.2) to calculate the % recovery for accuracy.

Acceptance criterion:

The difference between the % mean recovery and the theoretical (100%) must be ± 2.0% for all six injections (recovery should be 98.0 – 102.0%).

C.4 PRECISION

C.4.1 Repeatability

- Use data obtained from accuracy testing.
- Calculate the %RSD of the absorbance values of the six solutions.

Acceptance criterion:

The %RSD must be ≤ 2.0.

C.4.2 Reproducibility

- An independent analyst should prepare a standard solution, following the method as described in section B.5. The study must differ from that of the repeatability with regards to day, analyst and equipment.
- Use different UV spectrophotometers.
- Make use of different laboratories.
- Measure the absorbance of these solutions at 302 nm as determined in B.7.
- Calculate the %RSD of the for all the samples combined.

Acceptance criterion:

The %RSD must be ≤ 2.0 for the calculated values.

C.5 ROBUSTNESS

- Prepare a standard solution following the method as described in section B.5, obtaining 100% of the target concentration (use a standard solution prepared for accuracy testing).
- Measure the absorbance of this solution at 302 nm as determined in B.7 directly after preparation (day 1) and keep the solution under controlled conditions (on the bench at about 23ºC).
- Repeat the measurements on the initial solution on days 2 (24 hours) and 3 (48 hours).

Acceptance criterion:

The sample should show a maximum deviation of ≤ 1.0% from the initial value after 48 hours.

C.6 DETERMINATION OF

Use the accuracy data to calculate the value.

PART D: VALIDATION RESULTS, SUMMARY AND DISCUSSION

Table 2 Summary of validation results for UV-VIS spectrophotometry assay method

Parameter	Acceptance criteria	Results
Specificity	The spectrum of test solution must conform to that of the standard solution.	The spectrum of the test solution conformed to that of the standard solution and the solvent was used as blank (Figure 1).
Linearity & Range	The method should be linear over a range of 60 – 120% and the correlation coefficient $R^2 \geq 0.99$.	The method was linear over a range of 60 – 120% and $R^2 = 0.9999$ (Table 3 and Figure 2).
Accuracy	The difference between the % mean recovery and the theoretical (100%) must be ± 2.0% for all six solutions (% recovery should be 98.0 – 102.0%).	The average of the six solutions was 99.72% and the % recovery for all six solutions was between 98.8 – 100.8% (Table 4).
Precision		
A: Repeatability	The %RSD must be ≤ 1.0%.	%RSD = 0.7% (Table 4).
B: Reproducibility	The % difference between the laboratories should be ≤ 2.0%.	% Difference between laboratories = 0.2% (Table 5).
Robustness	The samples should show a maximum deviation of ≤ 1.0% from the initial value after 48 hours.	The samples showed a deviation of 0.3% from the initial value after 48 hours (Table 6).
Determination of	Use the accuracy data to calculate the value.	= 331.4 (Table 4).

D.1 SPECIFICITY

The spectrum recorded for the test solution (Figure 1) conformed to that of the standard solution. A maximum which can be used for identification purposes was obtained at 302 nm. Thus for identification purposes a scan from 275 – 325 nm would be sufficient to detect the analytical wavelength maximum at about 302 nm.

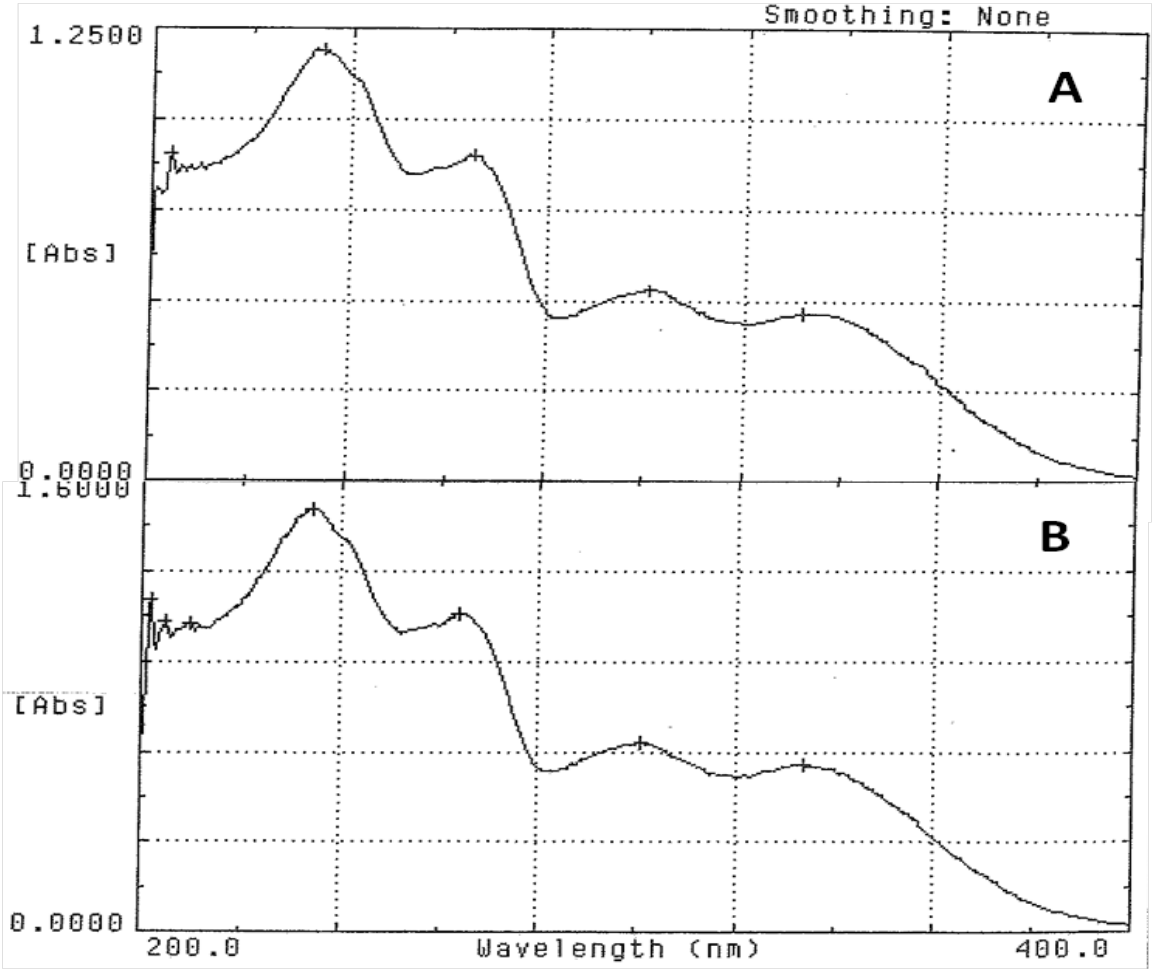


Figure 1 The UV spectra of lumefantrine standard solution (A) and test solution (B) recorded from 200 – 400 nm.

D.2 LINEARITY AND RANGE

Linearity and range results for lumefantrine are given in Table 3 and Figure 2.

This method is linear for the range between 60 and 120% and the correlation coefficient R^2 is 0.9999 as seen in Figure 2. The method is suitable for a single point calibration.

Table 3 The concentrations used and absorbances measured for lumefantrine linearity and range

Percentage (%) of target	Lumefantrine concentration (µg/ml)	Absorbance
60	12.09	0.4012
80	16.12	0.5349
90	18.14	0.6006
100	20.15	0.6688
110	22.17	0.7356
120	24.18	0.8057

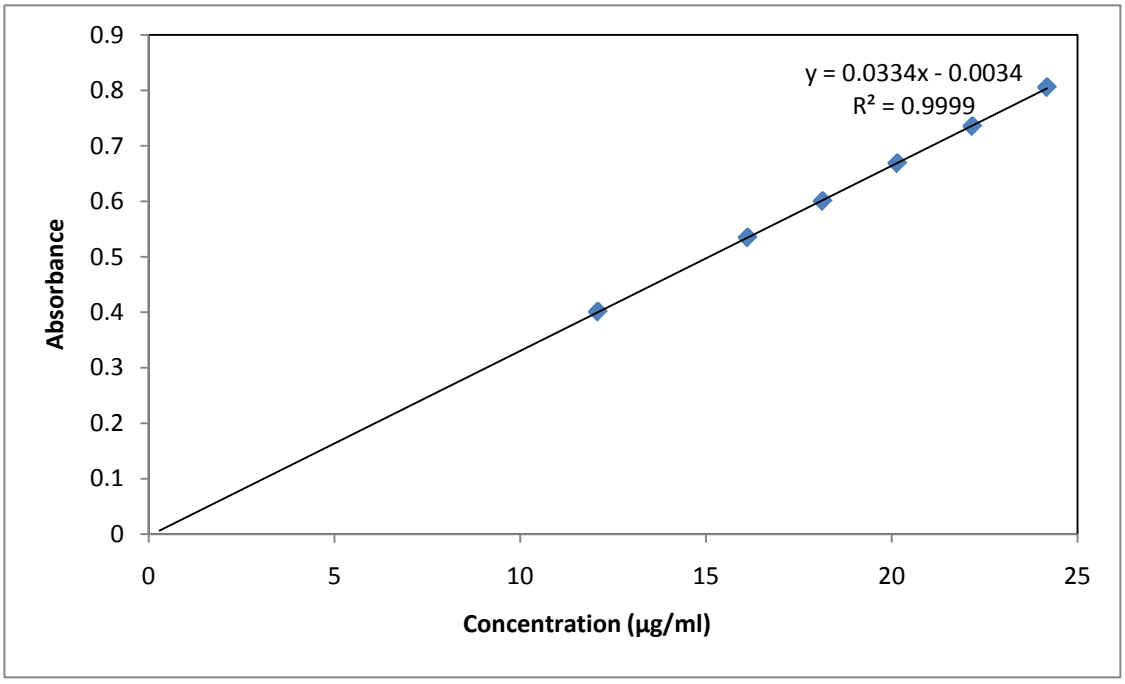


Figure 2 Linear regression graph for lumefantrine linearity and range.

D.3 ACCURACY

Results for accuracy of lumefantrine are given in Table 4, the % recovery for all six solutions were between 98.8 – 100.8% (acceptance criterion 98.0 – 102.0%).

Table 4 The concentrations used, absorbances measured and % recovery obtained for lumefantrine accuracy and repeatability tests

Sample	Mass (mg)	Actual concentration (µg/ml)	Absorbance	Experimental concentration (µg/ml)	Recovery (%)	
1	20.36	20.36	0.6752	20.32	99.8	331.6
2	20.31	20.31	0.6719	20.22	99.5	330.8
3	20.23	20.23	0.6779	20.40	100.8	335.1
4	20.12	20.12	0.6650	20.01	99.5	330.5
5	20.34	20.34	0.6680	20.10	98.8	328.4
6	20.15	20.15	0.6688	20.13	99.9	331.9
Average	**-**	**-**	**0.6711**	**-**	**99.72**	**331.4**
%RSD	**-**	**-**	**0.7%**	**-**	**0.7%**	**0.7%**

D.4 PRECISION

D.4.1 Repeatability

Repeatability results for lumefantrine are presented in Table 4 and were satisfactory with a %RSD of 0.7% (acceptance criterion ≤ 2.0%).

D.4.2 Reproducibility

An additional test solution (Table 5) was prepared by an independent analyst in another laboratory, using the same test sample (batch of lumefantrine) and a different UV spectrophotometer.

Table 5 Reproducibility results for the same API tested in different laboratories

Laboratories	Recovery (%)
Research Institute for Industrial Pharmacy (RIIP)	99.72
Centre for quality assurance of medicine (CENQAM)	99.51

D.5 ROBUSTNESS

The same sample was kept under controlled conditions in the laboratory (on a bench at a controlled temperature of about 23 °C) and measured on the same instrument on different days (Table 6).

Table 6 The stability of a 20.15 µg/ml lumefantrine solution for robustness test

Sample	Absorbance	% Recovery
Initial	0.6769	100.0
After 24 hours	0.6783	100.3
After 48 hours	0.6737	99.6
%RSD		0.3%

The aged sample showed a deviation of 0.4% from the initial after 48 hours and proved to be stable for at least 48 hours (acceptance criterion ≤ 1.0%).

D.6 DETERMINATION OF

The accuracy data were used to calculate the value, see Table 4.

The specific absorbance ( ) was calculated as 331.4. For identification purposes a variation of 5% is allowed resulting in a range of 314 and 348 as provided in the monograph.

PART E: CONCLUSION

An alternative assay method was developed for lumefantrine using UV spectrophotometry. This method was developed to include the identification test in the assay and to be effective for use in cases where reference standards are not readily available. This method is regarded as a fast, uncomplicated, effective and inexpensive identification and assay method.

The method proved to be specific and linear between a range of 60 – 120%. Accuracy was demonstrated, while precision testing proved the method to be repeatable and reproducible. Robustness with respect to stability of samples revealed samples to be stable for at least 48 hours at ambient conditions. The in methanol was determined as 331.4.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 4

APPENDIX E

Validation of a thin layer chromatographic method for a limit test for lumefantrine related substances

PART A: OBJECTIVE

The objective is to validate the thin layer chromatographic method for the detection of related substances (semi-quantitative) of lumefantrine active pharmaceutical ingredient. The evaluation and validation of this method will be done according to the ICH Guidelines on Validation of Analytical Procedures Q2(R1) (2005:3). Specificity, detection limit (DL) and robustness will be investigated.

PART B: RELATED SUBSTANCE TEST PROCEDURES

B.1 ORIGIN OF METHOD

Self-developed.

B.2 PRINCIPLE OF METHOD

The identification of lumefantrine related substances in lumefantrine test sample should inimitably be identified and semi-quantitatively detected (when present) by means of thin layer chromatography.

B.3 EQUIPMENT

- TLC chamber
- Aluminium TLC plate coated with silica gel R6[1] (Macherey-Nagel, Alugram® SIL G/UV_{254})
- Glass TLC plate coated with silica gel R6[1] (Macherey-Nagel, DC-Fertigplatten® SIL G-25/UV_{254})
- UV source with wavelength 254 nm
- Volumetric glassware (A grade, class 1)

B.4 REAGENTS AND SOLVENTS

- Lumefantrine primary reference standard (RS), provided by the manufacturer (Table 1)
- Lumefantrine test samples (Table 1)

[1] Silica gel 60 (UV 254), a white, homogeneous powder. Average pore size = 6 nm.
Composition: Silica gel (average particle size = 15 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg) (Ph.Int., 2008).

- Lumefantrine related substance A, provided by the manufacturer (Table 1)
- Lumefantrine related substances B and C (mixture of isomers), provided by the manufacturer (Table 1)
- Light petroleum ether R1 (boiling point: 40ºC - 60ºC) (Merck, AR grade)
- Ethyl acetate R (Merck, AR grade)
- Glacial acetic acid R (Merck, AR grade)
- Mobile phase: Prepare a mixture of 40 volumes light petroleum ether R1, 6 volumes of ethyl acetate R and 10 volumes of glacial acetic acid R.

Table 1 Lumefantrine reference standards and test samples used for validation of TLC related substances method

Description	Batch number	Manufacturing Company
Primary standard	C0189	Novartis
Lumefantrine test sample 1	070701	Tianjin Hi-tesion Bio & Chem Co., Ltd.
Lumefantrine test sample 2	090306	IFF, Iffect Chemphar (HK) Company Limited
Lumefantrine related substance A	802CB	Novartis
Lumefantrine related substance mixture B and C	6978E	Novartis

B.5 TEST SOLUTION (Solution 1)

Prepare a 10 mg/ml solution (1) of lumefantrine (test sample) in ethyl acetate R.

B.6 REFERENCE SOLUTIONS (Solutions 2 – 4)

Prepare the following solution using ethyl acetate R as solvent. For solution (2) dilute 1 ml of solution (1) to 100 ml with the solvent. For solution (3) dilute 3 ml of solution (2) to 10 ml with the solvent. For solution (4) dilute 2 ml of solution (2) to 20 ml with the solvent.

B.7 MEASUREMENT PROCEDURE

To a silica gel R6 plate separately apply 10 µl of each of the test and standard solutions, except solution 2. After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 10 - 15 cm in a pre-saturated chromatographic chamber. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram under UV light at 254 nm.

B.8 INTERPRETATION OF RESULTS

- Note the position, colour, size, intensity and calculate the Rf-values.
- In the chromatogram obtained with solution (1): Any spot other than the principal spot, is not more intense than that in the chromatogram obtained with solution (3) (0.3%) and not more than two such spots are more intense than that in the chromatogram obtained with solution (4) (0.1%).

PART C: VALIDATION PARAMETERS, TEST PROCEDURES AND ACCEPTANCE CRITERIA

C.1 SPECIFICITY

For specificity separate samples of the reference standard and the available impurities (related substances) were prepared and should show different Rf values for the different spots. Prepare a test solution (1) following the procedure for test solutions (according to section B.5), furthermore separately dissolve 2 mg of lumefantrine RS [reference solution (A)] and 2 mg of each of the lumefantrine related substances RS [reference solutions (B) and (C)] in 20 ml solvent. Spot 10 µl of each of these solutions (test and reference solutions) on a plate and follow the measurement procedure. Examine the plates visually. Note the Rf-values for all principle spots, description of the spots which includes shape, colour and intensity.

Acceptance criteria:

- Spots produced by solutions (A), (B) and (C) should be clearly visible under UV radiation light at 254 nm.
- Spots due to solutions (A), (B) and (C) must have different Rf values to allow clear separation, solution (C) should produce 2 spots.

C.2 DETECTION LIMIT

- For the validity of impurity testing, the minimum limit detected will be considered according to the lowest limit proposed which in this case is 0.1%. Standards with concentrations of 0.1% and 0.3% of the test solution should be tested and clearly visible in order for the test to be valid.

Acceptance criterion:

The spot obtained for the 0.1% solution should be clearly visible.

C.3 ROBUSTNESS

- Repeat the specificity test after 24 hours, using the initial test and reference solutions to determine stability of aged solutions.
- Two different analysts should do the test using both glass and aluminium silica gel R6 TLC plates each. Note the results Rf-values for all principle spots and description of the spots which includes shape, colour and intensity.

Acceptance criteria:

- The spots observed on the plates obtained from the aged solutions should be similar to those obtained from freshly prepared solutions.
- The aluminium and glass TLC plates should produce results corresponding to each other with regards to appearance, intensity and position (RRf).

PART D: SUMMARY AND DISCUSSION OF VALIDATION RESULTS

Table 2 Summary of validation results for the TLC related substances method

Parameter	Acceptance criteria	Results
Specificity	Spots produced by solutions (A), (B) and (C) should be clearly visible under UV light 254 at nm. Spots due to solutions (A), (B) and (C) must have different Rf values to allow clear separation, solution (C) should produce 2 spots.	The spots appeared as clearly visible, dark, round to oval shaped spots under UV light at 254 nm. Spots due to reference solutions (A), (B) and (C) had different Rf values and illustrated good separation (Figure 1).
Detection limit (DL)	The spot obtained for the 0.1% solution should be clearly visible.	The spot for 0.1% was clearly visible.
Robustness A: Stability of standard and test solutions after standing for 24 hours	The spots observed on the plates obtained from the aged solutions should be similar to those obtained from freshly prepared solutions.	The results produced by the aged sample corresponded to the freshly prepared sample with regards to appearance, intensity and position (Rf) and proved the solutions to be stable for at least 24 hours.
B: Use of glass and aluminium plates by two analysts	The aluminium and glass TLC plates should produce results corresponding to each other with regards to appearance, intensity and position (RRf).	Both the aluminium and glass TLC plates produced results acceptable for identification according to the acceptance criteria (Table 3 and 4).

D.1 SPECIFICITY

The spots produced by solutions (A), (B) and (C) appeared as clearly visible, dark, round to oval shaped spots under UV light at 254 nm. Spots due to reference solutions (A), (B) and (C) had different Rf values and illustrated good separation (Figure 1). The RRf of the principle spots were comparable (Table 3).

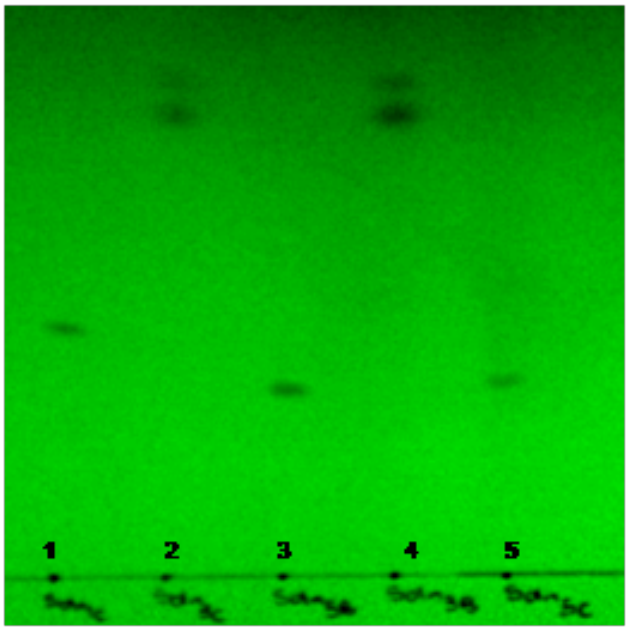


Figure 1 TLC plate obtained for specificity testing. Spot 1: lumefantrine primary standard [solution (A)], spots 2 and 4: lumefantrine related substances B and C [solution (C)] and spots 3 and 5 related substance A [solution (B)].

D.2 DETECTION LIMIT

The spot obtained for the solution with a concentration of 0.1% (the limit) with respect to the test solution is of acceptable visibility (Figure 2).

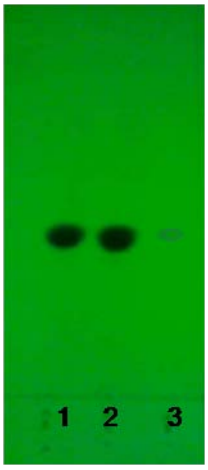


Figure 2 The TLC plate for detection limit testing, spot 1: standard solution, spot 2: test solution, spot 3: 0.1% dilution of the test solution.

D.3 ROBUSTNESS

The results produced by the aged test and reference solutions compared to the freshly prepared test and reference solutions with regards to appearance, intensity and position (Rf) of the spots and proved to be stable for at least 24 hours at ambient conditions. The test and reference solutions should be used within 24 hours after preparation. No comment with respect to the stability of these solutions is needed in the Ph.Int. monograph of lumefantrine API, since the period of 24 hours is sufficiently long for conducting the test.

The aluminium and glass TLC plates produced results concordant to each other with regards to appearance and intensity of the spots. The position (Rf values, Table 3 and 4) differed between the glass and aluminium plates however, this is not considered important – not being part of the acceptance criteria – since the test is always conducted using one single plate, be it an aluminium plate or a glass plate. The RRf values for the mixture of related substances B and C did not always reflect the clear separation since, although these two spots were separated, they were still near to each other.

Table 3 Results obtained for robustness test on glass plates

Glass plates	**Analyst A**			**Analyst B**		
	mm	Rf	RRf	mm	Rf	RRf
Mobile phase	136	1		127	1	
Lumefantrine	45	0.33	1	48	0.38	1
Related substance A	35	0.26	0.8	37	0.29	0.8
Related substance B	84	0.62	1.9	96	0.76	2.0
Related substance C	90	0.66	2.1	100	0.79	2.1

Table 4 Results obtained for robustness test on aluminium plates

Aluminium plates	Analyst A (Day 1)			Analyst B (After 24 hours)		
	mm	Rf	RRf	mm	Rf	RRf
Mobile phase	147	1		148	1	
Lumefantrine	45	0.31	1	50	0.34	1
Related substance A	25	0.17	0.6	32	0.22	0.7
Related substance B	131	0.89	2.9	114	0.77	2.3
Related substance C	138	0.94	3.0	121	0.82	2.4

PART E: CONCLUSION

A TLC method for the limit test of lumefantrine related substances has been developed and validated (summary of the validation results Table 2). Examination under UV light (254 nm) using silica gel R6 plates (HF 254) was effective for visualisation of the spots due to the known related substances. These related substances were provided by the manufacturer.

The method proved to be specific and robust with respect to either aluminium or glass TLC plates. The known related substances were adequately separated, and could be detected at a limit of 0.1% (which is the lowest limit for related substances) under UV light at 254 nm. The test and sample solutions proved to be stable for at least 24 hours at ambient conditions.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 4.

APPENDIX F

Validation of a titrimetric method for the assay of lumefantrine

PART A: OBJECTIVE

The objective was to validate a titration method for lumefantrine API assay. The evaluation and validation of this method will be done according to the ICH Guidelines on Validation of Analytical Procedures Q2(R1) (2005:3). Accuracy, precision (repeatability, intermediate precision), specificity, linearity and range will be investigated.

PART B: TITRIMETRIC TEST PROCEDURES

B.1 ORIGIN OF METHOD

Method from manufacturer supplied by the WHO.

B.2 PRINCIPLE OF METHOD

A potentiometric titration with perchloric acid in order to determine the content of lumefantrine.

B.3 EQUIPMENT

- Metrohm 785 DMP Titrino (Metrohm, Switzerland)
- Metrohm Solvatrode electrode (LiCl in ethanol)
- Glassware (A grade, class 1)

B.4 SAMPLES, REAGENTS AND SOLVENTS

- Lumefantrine primary reference standard (RS), provided by manufacturer (Table 1)
- Glacial acetic acid R1 (Merck, AR grade)
- Perchloric acid (0.1 mol/l) VS

Table 1 Lumefantrine reference standard and test sample used for validation of titrimetric assay method

Description	Batch number	Manufacturing Company
Primary standard	C0189	Novartis
Lumefantrine test sample	090306	IFF, Iffect Chemphar (HK) Company Limited

B.5 TEST SOLUTION FOR LUMEFANTRINE

Dissolve about 0.45 g of lumefantrine test sample, accurately weighed, in 50 ml of glacial acetic acid R1, stirring for about 15 minutes.

B.6 MEASUREMENT PROCEDURE

Titrate with perchloric acid (0.1 mol/l) VS, determine the end-point potentiometrically (as for non aqueous titrations, Method A, Ph.Int.). A glass electrode and a saturated calomel cell (containing *potassium chloride (350 g/l) TS*) are used (as reference electrode).

B.7 CALCULATION OF RESULTS

$$\text{Content of lumefantrine \%} = \frac{EP1 \times C01 \times C31 \times 100}{C00}$$

Where:

EP1 = Volume titre

C01 = Equivalence factor

(Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 52.89 mg of $C_{30}H_{32}Cl_3NO$)

C31 = Standardisation value of perchloric acid (normality)

C00 = Mass of sample weighed in mg

If the temperature (t_2) at which the titration is carried out differs from the temperature (t_1) at which the titrant was standardised, multiply the volume of the titrant required by [1 + 0.001(t_1 - t_2)] and calculate the result of the assay from the corrected volume.

PART C: VALIDATION PARAMETERS, TEST PROCEDURES AND ACCEPTANCE CRITERIA

The ICH guideline Q2(R1) (2005) will serve as reference for validation of this analytical method. The acceptance criteria for the specific validation parameters will be defined in the following sections.

C.1 SPECIFICITY

The non aqueous titration used for the assay is a non specific method and therefore no specificity testing was performed. However, either the TLC or HPLC limit tests for lumefantrine related substances should be performed along with the assay in order to support the assay by titration.

C.2 LINEARITY AND RANGE

Linearity and range will be evaluated simultaneously across a specific range of the analytical procedure.

- Prepare test solutions for lumefantrine following the method as described in (B.5) but using the following masses of the test sample: for solution (1) weigh about 0.36 g (80%); for solution (2) weigh about 0.405 g (90%); for solution (3) weigh about 0.45 g (100%); for solution (4) weigh about 0.495 g (110%); for solution (5) weigh about 0.54 g (120%).
- Carry out the measurement procedure (B.6) on these samples.
- Conduct a least squares linear regression analysis and calculate the correlation coefficient.

Acceptance criterion:

The method should be linear over a range of 80 – 120% with the correlation coefficient (R^2) ≥ 0.99.

C.3 ACCURACY

The accuracy will be assessed using a minimum of nine determinations over three concentration ranges (80%, 100% and 120%) that cover the range of analysis.

- Prepare 3 solutions following the method as described under test solution (B.5) preparation, obtaining 80% of the target mass (weighing 0.36 g instead of 0.45 g).
- Prepare 3 solutions following the method as described under test solution (B.5) preparation, obtaining 100% of the target mass.
- Prepare 3 solutions following the method as described under test solution (B.5) preparation, obtaining 120% of the target mass (weighing 0.54g instead of 0.45g).
- Carry out the measurement procedure (B.6) on these solutions.
- Calculate the mean recovery and the %RSD.

Acceptance criterion:

The mean recovery must be ± 1% of the theoretical 100% in all three series.

C.4 PRECISION

C.4.1 Repeatability

- Use data obtained from accuracy testing.
- Calculate the %RSD of the nine determinations.

Acceptance criterion:

The %RSD must be ≤ 1%.

C.4.2 Intermediate precision

- Different analysts conduct the test as described in B.5 (in triplicate) on different days, using the same batch of lumefantrine test sample.
- Carry out the measurement procedure on all solutions.
- Calculate the %RSD.

Acceptance criterion:

The %RSD must be ≤ 2% for each study.

PART D: VALIDATION RESULTS, SUMMARY AND DISCUSSION

Table 2 Summary of validation results for assay by titration

Parameter	Acceptance criteria	Results
Linearity & Range	The method should be linear over a range of 80 – 120% with the correlation coefficient (R^2): ≥ 0.99.	The method was linear over a range of 80 – 120% and R^2 = 0.9993.
Accuracy	The mean recovery must be ± 2% of the theoretical 100% in all three series.	The mean recovery was between 99.50 and 101.50%, with an average of 100.5% in 9 samples.
Precision		
A: Repeatability	The %RSD must be ≤ 2.0%.	The %RSD = 0.5%.
B: Intermediate precision	The %RSD must be ≤ 2.0%.	The %RSD = 0.4%.

D.1 SPECIFICITY

The testing of the related substances along with the assay is also part of the monograph specifications. In the HPLC test for related substances on this sample no peaks were detected for the related substances (see Figure 1).

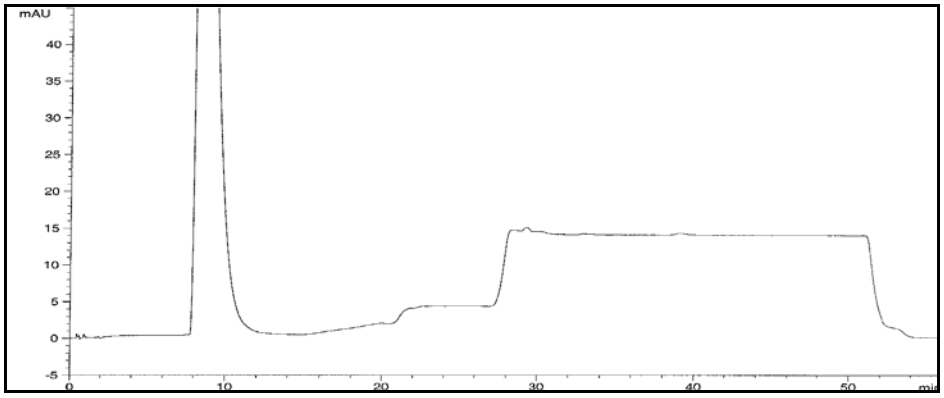


Figure 1 HPLC chromatogram of lumefantrine test sample, indicating no related substances.

D.2 LINEARITY AND RANGE

Linearity and range results for lumefantrine assay are given in Table 3 and Figure 2.

Table 3 The concentrations used and titre measured for lumefantrine linearity and range

Percentage of target (%)	Mass of test sample weighed (mg)	Titre (ml)
80	361.27	7.2
90	406.55	8.1
100	451.30	9.0
110	490.55	9.8
120	541.49	10.7

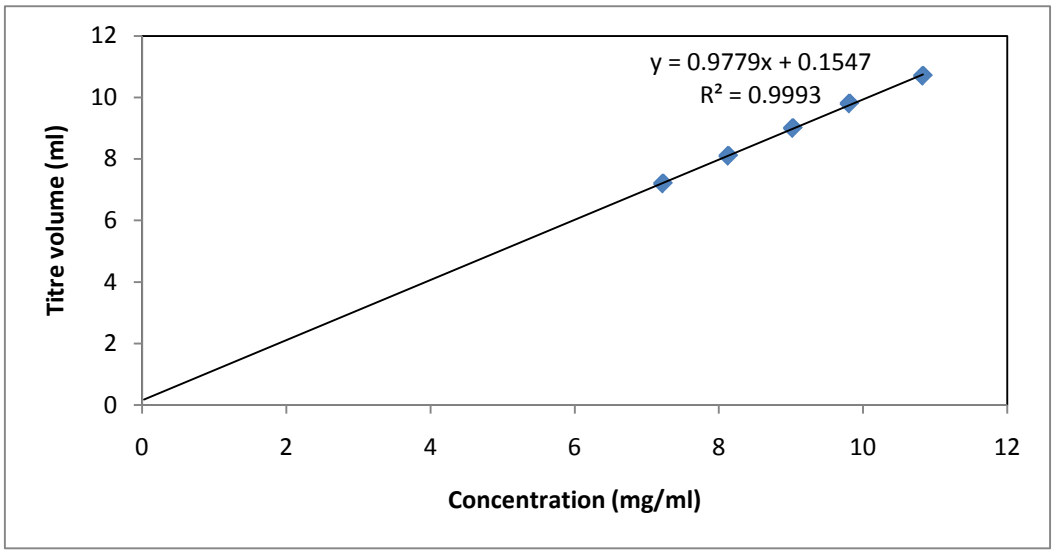


Figure 2 Linear regression graph for lumefantrine titrimetric assay: linearity and range.

This method is linear for the range between 80 and 120% and the correlation coefficient (R^2) is 0.9993 as seen in Figure 3. The method is suitable for a single point calibration.

D.3 ACCURACY

The mean recovery is 100.5%, all samples are within ± 2% of the theoretical 100% in all three series (Table 4).

Table 4 Accuracy and repeatability results for lumefantrine titration assay

Sample	Mass (mg)	Concentration mg/ml	Titre (ml)	% Recovery
1	361.27	7.225	7.2	100.35
2	361.59	7.232	7.2	100.26
3	362.14	7.243	7.3	101.50
4	451.30	9.026	9.0	100.41
5	450.12	9.002	9.0	100.68
6	450.54	9.011	9.0	100.58
7	541.49	10.830	10.7	99.50
8	541.59	10.832	10.8	100.41
9	541.55	10.831	10.8	100.41
Average	**-**			**100.5**
%RSD	**-**			**0.5%**

The mean recovery must be ± 2% of the theoretical 100% in all three series and is calculated as 100.5%.

D.4 PRECISION

D.4.1 Repeatability

The data obtained for the repeatability test is summarised in Table 4 (with accuracy).
The %RSD must be ≤ 1% and is calculated as 0.5%.

D.4.2 Intermediate precision

The data for intermediate precision testing is presented in Table 5. Samples 1-3 were tested by analyst A on day 1 and samples 4-6 were tested by analyst B on day 2. The %RSD between all samples were 0.4%, thus within acceptance criterion limits.

Table 5 Intermediate precision results for lumefantrine assay titration on one sample

Sample	Mass (mg)	Titre (ml)	% Recovery
1	456.76	9.1	100.16
2	457.35	9.1	100.00
3	451.84	8.9	99.79
4	451.30	9.0	100.41
5	450.12	9.0	100.68
6	450.54	9.0	100.58
Average			**100.3**
%RSD			**0.4%**

The %RSD of the 6 preparations at 100% target strength must be ≤ 1% and is calculated as 0.4%.

D.5 SUPPLEMENTARY TESTING

Assay testing was performed on the same test sample, using both the UV-VIS and titration methods. The results obtained are summarised in Table 6.

The %RSD between the averages of the UV (additional method validated Appendix D) and titration methods was 0.15%. The difference between the highest and lowest value obtained was 0.3% which indicates that the methods for assay with these two different techniques produced similar results.

Table 6 Lumefantrine UV and titration assay results and %RSD

Sample	UV Assay	Titre Assay
Sample 1	100.3%	100.2%
Sample 2	100.1%	100.0%
Average	**100.2%**	**100.1%**

PART E: CONCLUSION

A titrimetric method for the assay of lumefantrine API has been developed and validated.

The method proved to be linear and repeatable within a range of 80 – 120% and specificity was confirmed by the complimentary HPLC test for related substances. Accuracy was proven and the results for two different analysts were comparable. Supplementary tests produced similar results for the UV-VIS and titration assay methods.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 4.

APPENDIX G

Validation of a thin layer chromatographic method for the identification of artemether and lumefantrine in artemether/lumefantrine tablets

PART A: OBJECTIVE

The objective is to validate the thin layer chromatographic method for identification of artemether and lumefantrine APIs in artemether/lumefantrine tablets. In order to evaluate and validate this method according to the ICH Guidelines on Validation, ICH Q2(R1) (2005:3), the specificity and robustness (even though robustness is not a requirement by the ICH guidelines) of this method will be investigated.

PART B: IDENTIFICATION TEST PROCEDURES

B.1 ORIGIN OF METHOD

Method for artemether/lumefantrine 20/120 mg tablets, from manufacturer supplied by the WHO.

B.2 PRINCIPLE OF METHOD

The identification of the artemether and lumefantrine APIs should inimitably be confirmed by means of thin layer chromatography. This test is to be done in conjunction with the HPLC test for ID, to fulfil the ICH Q6A (1999:6) Guideline requirements for specificity.

B.3 EQUIPMENT

- TLC chamber
- Aluminium TLC plate coated with silica gel R5[1] (Macherey-Nagel, Alugram® SIL G)
- Glass TLC plate coated with silica gel R5[1] (Macherey-Nagel, DC-Fertigplatten® SIL G-25)
- Aluminium TLC plate coated with silica gel R6[2] (Macherey-Nagel, Alugram® SIL G/UV_{254})
- Glass TLC plate coated with silica gel R6[2] (Macherey-Nagel, DC-Fertigplatten® SIL G-25/UV_{254})
- UV source with wavelength 254 nm
- Volumetric glassware (A grade, class 1)

[1] Silica gel 60, a white, homogeneous powder. Average pore size = 6 nm (Ph.Int., 2008)
[2] Silica gel 60 (UV 254), a white, homogeneous powder. Average pore size = 6 nm.
Composition: Silica gel (average particle size = 15 µm) containing a fluorescent indicator having an optimal intensity at 254 nm (about 15 g/kg) (Ph.Int., 2008).

B.4 REAGENTS, SOLVENTS AND SOLUTIONS

- Artemether primary reference standard (RS), provided by the manufacturer (Table 1)
- Lumefantrine primary reference standard (RS), provided by the manufacturer (Table 1)
- Artemether/lumefantrine test samples (Table 1)
- Methanol R (Merck, AR grade)
- Acetone R (Merck, AR grade)
- Water R
- Sulphuric Acid R (Merck, AR grade)
- Light petroleum ether R1 (boiling point: 40ºC - 60ºC) (Merck, AR grade)
- Ethyl acetate R (Merck, AR grade)
- Glacial acetic acid R (Merck, AR grade)
- Iodine pellets (Merck, AR grade)
- Mobile phase: Prepare a mixture of 40 volumes light petroleum ether R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R.
- *Sulfuric acid/methanol TS*: Cool separately 10 ml of sulfuric acid TS (~1760 g/l) and 90 ml of methanol AR. Carefully add the acid to the methanol AR, keeping the solution as cool as possible, and mix gently (Ph.Int., 2008).

Table 1 Reference standards and test sample used for validation of TLC identification method

Description	Batch number	Manufacturing Company
Lumefantrine primary standard	C0189	Novartis
Artemether primary standard	C0015	Novartis
Artemether/lumefantrine 20/120 mg tablets	X1435	Novartis (Coartem® 20/120)

B.5 TEST SOLUTION

Prepare the following solution using acetone R as solvent: Weigh and powder 20 artemether/lumefantrine 20/120 mg tablets. Shake a quantity of the powder containing 10 mg of artemether (about 60 mg of lumefantrine) for 5 minutes with 10 ml solvent. Filter (discarding the first few ml of the filtered solution) and use the filtrate.

B.6 STANDARD SOLUTION

Prepare a standard solution containing 1 mg of artemether RS and 6 mg of lumefantrine RS per ml in acetone R.

B.7 MEASUREMENT PROCEDURE

The following two methods are basically identical in procedure, but are however different in detection technique.

B.7.1 To a silica gel R6 plate separately apply 10 µl of each of the test and standard solutions. After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 10 - 15 cm in a saturated chromatographic chamber, using the mobile phase as described under B.4. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram under UV light at 254 nm.

Spray the plate with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight.

The principle spot obtained with the test solution detected under UV light at 254 nm (before spraying) should correspond to that of lumefantrine obtained with the standard solution in position (Rf), appearance and intensity. The principle spot obtained with the test solution after spraying and heating should correspond to that of artemether obtained with the standard solution in position (Rf), appearance and intensity.

B.7.2 To a silica gel R5 plate separately apply 10 µl of each of the test and standard solutions. After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 10 - 15 cm in a saturated chromatographic chamber, using the mobile phase as described under B.4. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Spray the plate with sulfuric acid/methanol TS. Heat the plate for 10 minutes at 140°C. Allow the plate to cool down and expose it to iodine vapour for 20 minutes. Examine the chromatogram immediately in daylight.

The two principle spots obtained with the test solution should correspond to that obtained with the standard solution in position (Rf), appearance and intensity.

B.8 INTERPRETATION OF RESULTS

Examine the plate visually. Note the position, colour, size and intensity of the spots and calculate the Rf-values.

PART C: VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

The ICH guideline (ICH Q2(R1), 2005:1-17) will serve as reference for the validation of this analytical method. Both methods B.7.1 and B.7.2 will simultaneously be done on R6 plates for validation. It is apparent from B.7.1 and B.7.2 that the same plate used in B.7.1 can be exposed to iodine (B.7.2) and thus go through all the visualisation methods.

C.1 SPECIFICITY

Prepare test and standard solutions following the procedures as described in B.5 and B.6. Follow the measurement procedures as described in B.7.1 and B.7.2. Also prepare a "placebo solution" by shaking an analytical placebo mixture[1] equivalent to the mass of one tablet with 20 ml of acetone, followed by filtration. For qualitative composition of placebo see summary of product characteristics of Riamet® (the name used by Novartis in the ICH region for the equivalent of Coartem®).

Acceptance criteria:

The principle spot obtained in the chromatogram with the test solution detected under UV light at 254 nm (before spraying) should correspond to the principle spot of the standard solution representing lumefantrine, in position (Rf), appearance and intensity (as described in B.7.1).

The principle spot obtained by the test solution after spraying and heating corresponds to the principle spot in the standard solution representing artemether, in position (Rf), appearance and intensity (as described in B.7.1).

The two principle spots detected from the test solution after exposure to iodine vapour should correspond to those of the standard solution, representing artemether and lumefantrine, in position (Rf), appearance and intensity (as described in B.7.2).

Any spots detected from the placebo solution should not interfere with that detected from the standard solution.

C.2 ROBUSTNESS

- Allow the test solution prepared for specificity testing to stand on the shelf for 48 hours (to determine stability of test solutions).
- Apply the aged solutions, a freshly prepared standard solution (B.6) and a freshly prepared test solution (B.5) to both glass and aluminium silica gel R6 TLC plates. Follow the measurement procedures (B.7.1 and B.7.2).

Acceptance criteria:

- The principle spots obtained with the freshly prepared and aged solutions should be correspond with regards to appearance, intensity and position (Rf) to demonstrate the stability of standard and test solutions over a period of 48 hours, using all three detection methods. The Rf values of the aluminium and glass plates may differ.

[1] The analytical placebo consists of the following: polysorbate 80 (100 mg), hypromellose (100 mg), microcrystalline cellulose (500 mg), colloidal anhydrous silica (50 mg), croscarmellose sodium (100 mg) and magnesium stearate (60 mg).

PART D: SUMMARY AND DISCUSSION OF VALIDATION RESULTS

Table 2 Summary of validation results for TLC identification method

Parameter	Acceptance criteria	Results
Specificity	The principle spot obtained in the chromatogram with the test solution detected under UV light at 254 nm (before spraying) should correspond to the principle spot of the standard solution representing lumefantrine, in position (Rf), appearance and intensity.	The principle spot of the test solution detected under UV light at 254 nm (before spraying) corresponded to the principle spot of the standard solution representing lumefantrine, in position (Rf), appearance and intensity (Figure 1).
	The principle spot obtained with the test solution after spraying and heating corresponds to the principle spot obtained with the standard solution representing artemether, in position (Rf), appearance and intensity.	The principle spot obtained with the test solution after spraying and heating corresponded to the principle spot obtained with the standard solution representing artemether, in position (Rf), appearance and intensity.
	The two principle spots detected from the test solution after spraying and exposure to iodine vapour should correspond to those of the standard solution in position (Rf), appearance and intensity.	The two principle spots detected from the test solution after spraying and exposure to iodine vapours corresponded with those of the standard solution in position (Rf), appearance and intensity (Figure 1).
	Any spots detected from the placebo solution should not interfere with that detected with the standard solution.	No spots were detected with the placebo solution.
Robustness	The principle spots obtained with the freshly prepared and aged solutions should correspond with regards to appearance, intensity and position (Rf) using all three detection methods.	The results obtained after solutions stood for 48 hours corresponded with those of the freshly prepared solutions for all three detection methods.
	The Rf values of the aluminium and glass plates may differ.	The Rf values of the aluminium and glass plates differed significantly (Table 4).

D.1 SPECIFICITY

Table 3 Rf values obtained for artemether and lumefantrine standards and test sample (aluminium plate)

	Artemether		Lumefantrine	
	mm	Rf	mm	Rf
Mobile phase	141		141	
Standard	114	0.81	27	0.19
Sample	113	0.80	26	0.18

The principle spot obtained with the test solution according to all three detection methods corresponded to the principle spot obtained with the standard solution representing artemether and lumefantrine, in position (Rf), appearance and intensity (Figure 1).

No spots were detected from the placebo solution.

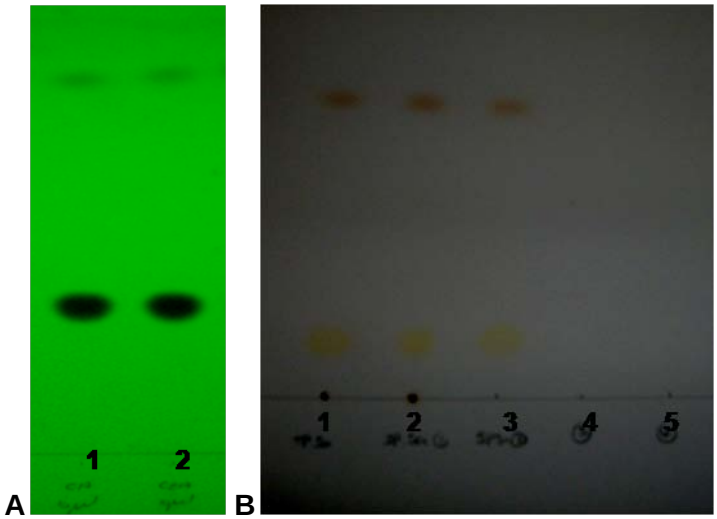


Figure 1 Identification by means of TLC:
The plate of a sample after treatment with *sulfuric acid in methanol TS* and exposure to iodine under UV light at 254 nm.
Plate A (under UV light at 254 nm, identifying lumefantrine): spot 1: artemether and lumefantrine RS solution, spot 2: artemether/lumefantrine tablet test solution.
Plate B (in daylight): spot 1: artemether/lumefantrine powder for suspension[1], spot 2: artemether/lumefantrine tablet, spot 3: artemether and lumefantrine RS solution, spot 4: analytical placebo (powder for suspension), spot 5: analytical placebo (tablets).

[1] The same plate was used for the powder for suspension specificity.

D.2 ROBUSTNESS

For the robustness test, both aluminium and glass plates were used. A freshly prepared standard solution and a freshly prepared test solution, as well as an aged test solution (48 hours at ambient conditions) were used. The data in Table 4 show little variation between the Rf value of the lumefantrine spots and no variation for artemether spots of the standard and test solutions on the aluminium plates. Similarly, little variation for lumefantrine and artemether exists between the spots of the standard and test solutions on the glass plates.

The aluminium plate (artemether Rf≈ 0.83; lumefantrine Rf ≈ 0.1 9) and glass plate (artemether Rf ≈ 0.64; lumefantrine Rf ≈ 0.26) showed significant differences in the Rf values of the artemether and lumefantrine spots. However, this is not considered important – being not part of the acceptance criteria – since the test is always conducted using one single plate, be it an aluminium plate or a glass plate.

Table 4 Rf values of artemether and lumefantrine calculated on glass and aluminium plates

Artemether	**Glass plate**		**Aluminium plate**	
	mm	Rf	mm	Rf
Mobile phase	129		134	
Sample (0 hours)	83	0.64	111	0.83
Sample (48 hours)	84	0.65	111	0.83
Standard	83	0.64	111	0.83
Lumefantrine	**Glass plate**		**Aluminium plate**	
	mm	Rf	mm	Rf
Mobile phase	129		134	
Sample (0 hours)	34	0.26	25	0.19
Sample (48 hours)	35	0.27	24	0.18
Standard	34	0.26	25	0.19

There was no practical difference between spots of the freshly prepared sample and the aged sample with respect to appearance, intensity, colour and position. The test and standard solutions should be used within 48 hours after preparation. No comment with respect to the stability of these solutions is needed in the Ph.Int. monograph of artemether/lumefantrine tablets, since a period of 24 hours is sufficiently long for conducting the test.

PART E: CONCLUSION

A TLC method for the identification of artemether and lumefantrine in artemether/lumefantrine tablets has been developed and validated. According to the general requirements of the Ph.Int., two methods were developed to visualise the artemether and lumefantrine spots. If the test laboratory is equipped with a UV lamp for TLC (254 nm), the first method would be the visualisation method of choice. If this is not the case, the second visualisation method could be used.

The TLC method proved to be specific and robust for both artemether and lumefantrine APIs with regards to aluminium or glass TLC plates. The excipients did not interfere in the test, and the standard and sample solutions were stable for at least 48 hours at ambient conditions.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 5.

APPENDIX H

Validation of a thin layer chromatographic method for a limit test of artemether related substances in artemether/lumefantrine tablets

PART A: OBJECTIVE

The objective is to validate the thin layer chromatographic method for the limit test of artemether related substances in artemether/lumefantrine tablets. In order to evaluate and validate this method according to the ICH Guidelines on Validation, ICH Q2(R1) (2005:3), specificity, detection limit and robustness of this method will be investigated.

PART B: IDENTIFICATION TEST PROCEDURES

B.1 ORIGIN OF METHOD

Method for artemether/lumefantrine 20/120 mg tablets, from manufacturer supplied by the WHO.

B.2 PRINCIPLE OF METHOD

The artemether related substances in artemether/lumefantrine tablets should inimitably be identified and semi-quantitatively detected (when present) by means of thin layer chromatography.

B.3 EQUIPMENT

- TLC chamber
- Aluminium TLC plate coated with silica gel R5 (Macherey-Nagel, Alugram® SIL G)
- Volumetric glassware (A grade, class 1)

B.4 REAGENTS, SOLVENTS[1] AND SOLUTIONS

- Artemether primary reference standard (RS), provided by the manufacturer (Table 1)
- Artenimol[2] primary reference standard (RS), provided by the manufacturer (Table 1)
- α - artemether primary reference standard (RS), provided by the manufacturer (Table 1)
- Artemether/lumefantrine test samples (Table 1)
- Lumefantrine primary reference standard (RS), provided by the manufacturer (Table 1)
- Ethanol R (Merck, AR grade)
- Acetonitrile R (Merck, AR grade)

[1] Grade of solvents (R) as defined in The International Pharmacopoeia.
[2] Also commonly known as Dihydroartemisinin.

- Water R
- Vanillin R (Merck, AR grade)
- Sulfuric Acid R (Merck, AR grade)
- Light petroleum ether R1 (boiling point: 40ºC - 60ºC) (Merck, AR grade)
- Ethyl acetate R (Merck, AR grade)
- Glacial acetic acid R (Merck, AR grade)
- Mobile phase: Prepare a mixture of 40 volumes light petroleum ether R1, 10 volumes of ethyl acetate R and 5 volumes of glacial acetic acid R.
- *Vanillin/Sulfuric acid TS2*: Dissolve 1 g of vanillin R in sufficient ethanol (~750 g/l) TS to produce 100 ml, carefully add, drop by drop, 2 ml of sulfuric acid (~1760 g/l) TS.

  Note: *Vanillin/sulfuric acid TS2* must be used within 48 hours.

Table 1 Reference standards and test sample used for validation of TLC method for limit test of artemether related substances

Description	Batch number	Manufacturing Company
Lumefantrine RS	C0189	Novartis
Artemether RS	C0015	Novartis
Artenimol RS (impurity B)	38276	Novartis
α – artemether RS (impurity D)	543B0	Novartis
Artemether/lumefantrine 20/120 mg tablets	X1435	Novartis (Coartem® 20/120)

B.5 TEST SOLUTION[1] (Solution 1)

Prepare the following solution using equal volumes of acetonitrile R and water R as solvent. Weigh and powder 20 artemether/lumefantrine 20/120 mg tablets. To a quantity of the powder containing 100 mg of artemether add 20 ml solvent, sonicate for 15 minutes and centrifuge. Filter a portion of the supernatant through a 0.45 µm filter, discarding the first few ml of the filtrate. Use the filtrate.

[1] Protect all solutions from light, also during chromatography.

B.6 STANDARD SOLUTIONS[1] (Solutions 2 – 7)

For solution (2) dissolve 5 mg of each of artemether RS, artenimol RS and α-artemether RS in 50 ml of the solvent. For solution (3) dilute 2.0 ml of solution (2) to 20 ml with the solvent (0.2%). For solution (4) dilute 3.0 ml of solution (2) to 20 ml with the solvent (0.3%). For solution (5) dilute 5.0 ml of solution (2) to 20 ml with the solvent (0.5%). For solution (6) dilute 1.0 ml of solution (2) to 2 ml with the solvent (1.0%). For solution (7) dilute 3.0 ml of solution (2) to 4 ml with the solvent (1.5%).

B.7 MEASUREMENT PROCEDURE[1]

To a silica gel R5 plate separately apply 20 µl of each of the test and standard solutions, except solution (2)[2]. After application allow the spots to dry for 15 minutes in a current of cool air. Develop over a path of 12 cm in a pre-saturated chromatographic chamber. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in vanillin/sulfuric acid TS2. Heat the plate for 10 minutes at 140°C. Examine the chromatogram in daylight. Note the Rf-values for all principle spots and the description of the spots which include appearance and intensity.

Artemether and related substances have the following Rf values: impurity A about 0.25; impurity B (artenimol) about 0.3; impurity C about 0.35; impurity D (α-artemether) about 0.4 and artemether about 0.55. See names and structures of impurities in Figure 1.

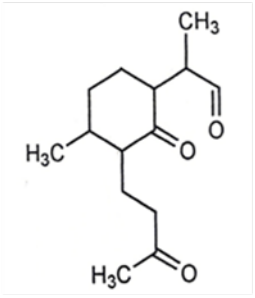
B.8 INTERPRETATION OF RESULTS

In the chromatogram obtained with solution (1):

- any spot corresponding in Rf value to impurity A is not more intense than the spot corresponding to artemether obtained with solution (7) (1.5%),
- any spot corresponding in Rf value to artenimol (impurity B) is not more intense than the spot corresponding to artenimol obtained with solution (6) (1.0%),
- any spot corresponding in Rf value to impurity C is not more intense than the spot corresponding to artemether obtained with solution (5) (0.5%),
- any spot corresponding in Rf value to α-artemether (impurity D) is not more intense than the spot corresponding to α-artemether obtained with solution (4) (0.3%),
- the spot of any other impurity is not more intense than the spot corresponding to artemether obtained with solution (3) (0.2%). Disregard any spot remaining at the point of application.

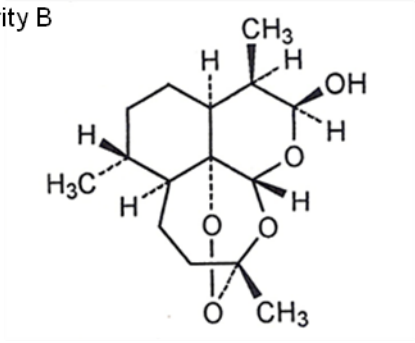
[1] Protect all solutions from light, also during chromatography.
[2] Solution (2) is not part of the measurement procedure, though is used in the validation study (specificity).

Impurity A



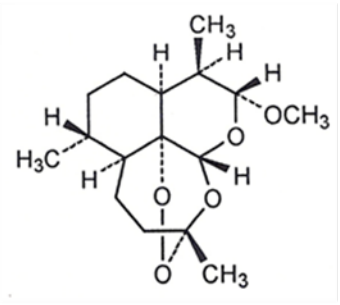
2-[4-methyl-2-oxo-3-(3-oxobutyl)cyclohexyl]propanal

Impurity B



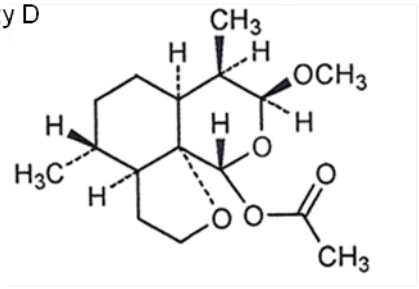
*(3R,5aS,6R,8aS,9R,10S,12R,12aR)-3,6,9-trimethyldecahydro-3,12-epoxy-12H-pyrano[4,3-j]-1,2-*benzodioxepin-10-ol (artenimol, dihydroartemisinin)

Impurity C



*(3aS,4R,6aS,7R,8S,10R,10aR)-8-methoxy-4,7-dimethyloctahydro-2H-furo[3,2-i][2]benzopyran-10-*yl acetate

Impurity D



(3R,5aS,6R,8aS,9R,10R,12R,12aR)-10-methoxy-3,6,9-trimethyldecahydro-3,12-epoxy-12Hpyrano[4,3-j]-1,2-benzodioxepine (α-artemether)

Figure 1 Names and structures of artemether impurities (taken from the monograph, Ph.Int., 2008).

PART C: VALIDATION PARAMETERS, TEST PROCEDURES AND ACCEPTANCE CRITERIA

The ICH guideline (ICH Q2(R1), 2005:1-13) will serve as reference for the validation of this analytical method.

Due to the unavailability of impurity A and impurity C, the Rf values from the data of the manufacturer will be used and will be confirmed by the Rf values obtained for the other two available related substances available (impurity B and impurity D).

C.1 SPECIFICITY

Prepare a test solution (1) following the procedure for test solutions (according to section B.5). Prepare a standard solution (2) following the procedure for standard solutions (B.6). Furthermore prepare a solution containing 60 mg lumefantrine RS in 2 ml solvent (solution (A)) and a "placebo solution"[1] (solution (B)), by sonicating an analytical placebo mixture equivalent to the mass of 1 tablet in 4 ml of solvent for 15 minutes. Filter a portion of the supernatant through a 0.45 µm filter, discarding the first 1 ml of the filtrate. Use the filtrate to spot 20µl of each of these solutions on a plate and follow the measurement procedure as described in section B.7.

Acceptance criteria:

- Solution (2) should produce three spots with different Rf values to allow clear separation.
- Spots produced by solutions (1) and (2) should be visible in daylight, solutions (A) and (B) should preferably show no spot.
- Spots due to solutions (A) and (B) should show no interference with any spot produced by solution (2).

C.2 DETECTION LIMIT

For the validity of related substances testing, the minimum limit detected will be considered according to the lowest limit proposed which in this case is 0.2% for any spot other than that of impurities A, B, C and D. Prepare a standard solution with a concentration of 0.1% (0.005 mg/ml) with respect to solution (1), by diluting 1 ml of solution (2) to 20 ml with solvent and follow the measurement procedure as described in section B.7.

Acceptance criterion:

- The spot obtained with a solution with a concentration of 0.005 mg/ml (0.1% of the test solution) should be clearly visible. This will ensure that the impurity with the lowest limit (0.2%) will be detected.

[1] The analytical placebo consists of the following: polysorbate 80 (100 mg), hypromellose (100 mg), microcrystalline cellulose (500 mg), colloidal anhydrous silica (50 mg), croscarmellose sodium (100 mg) and magnesium stearate (60 mg).

C.3 ROBUSTNESS

Store solutions (1) and (2) prepared for the specificity test for 48 hours (protected from light). After 48 hours prepare a fresh test solution (B.5) and a standard solution (B.6). Spot the aged and freshly prepared solutions together and follow the measurement procedure as described in section B.7.

Acceptance criterion:

- The spots of the of the aged solutions should compare with that of the freshly prepared solutions with regards to appearance, intensity and position (Rf).

C.4 SYSTEM SUITABILITY

For system suitability spot standard solution (3), following the measurement procedure in section B.7.

Acceptance criterion:

- The test is not valid unless the chromatogram obtained with standard solution (3) shows three spots which are clearly separated.

PART D: SUMMARY AND DISCUSSION OF VALIDATION RESULTS

Table 2 Summary of validation results for TLC of artemether related substances method

Parameter	Acceptance criteria	Results
Specificity	Solution (2) should produce three spots with different Rf values to allow clear separation. Spots produced by solutions (1) and (2) should be clearly visible in daylight, solutions (A) and (B) should preferably show no spot. Spots due to solutions (A) and (B) should show no interference with any spot produced by solution (2).	Solution (2) produced three spots with different Rf values. Spots produced by solutions (1) and (2) were clearly visible in daylight. Solutions (A) and (B) showed no spots. Solutions (A) and (B) showed no spots, thus no interference to any spot produced by solutions (1) and (2).
Detection limit	A standard with a concentration of 0.005 mg/ml (0.1% of the test solution) should be tested and the spot visible.	The spot for the 0.1% solution was visible.
Robustness	The spots of the aged samples should compare with that of the freshly prepared sample with regards to appearance, intensity and position (Rf).	The spots obtained for the aged samples (after 48 hours) were comparable to those of the freshly prepared samples.
System suitability	The test is not valid unless the chromatogram obtained with standard solution (3) shows three spots which are clearly separated.	The chromatogram obtained with standard solution (3) showed three spots which were clearly separated.

D.1 SPECIFICITY

Spots produced by solutions (1) and (2) were visible under daylight, solutions (A) and (B) showed no spots, therefore no interference to any spot produced by solutions (1) and (2). Solution (2) produced three spots with different Rf values and thus the method allowed clear separation.

Table 3 Rf values of artemether and related substances obtained in the test sample and standards for specificity

Substance	mm	Rf
Mobile phase	120	
Artemether	66	0.55
Impurity C[1]	42	0.35
Artenimol	36	0.30
α-artemether	48	0.40

The Rf values given in the proposed Ph.Int. method are as follows: impurity A about 0.25; impurity B (artenimol) about 0.3; impurity C about 0.35; impurity D (α-artemether) about 0.4; artemether about 0.55. The proposed Rf values are similar to those given in Table 3.

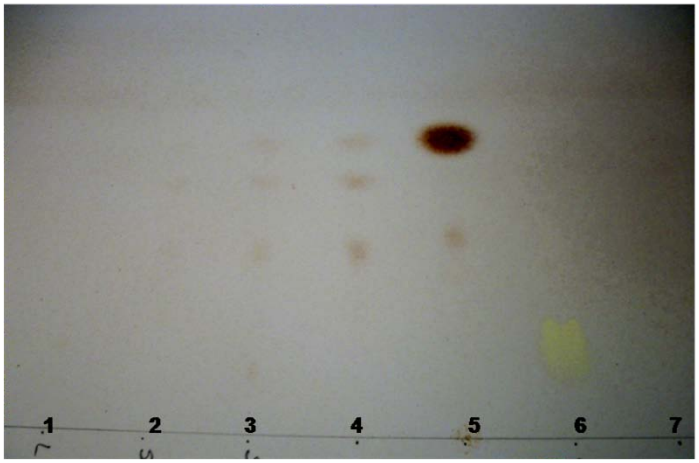


Figure 2 Spot 1: solution (A), spot 2: 0.1% solution (for detection limit), spot 3: solution (3) (system suitability), spot 4: solution (2) for specificity, spot 5: test solution (1) for specificity, spot 6: lumefantrine in ethyl acetate[2], spot 7: solution (B).

D.2 DETECTION LIMIT

Upon visual inspection the spot for the solution with a concentration of 0.005 mg/ml (0.1% of the test solution) was visible (spot 2 in Figure 2, not clear on the photo, however acceptably visible in daylight). The method is thus sensitive enough to detect the related substances at a concentration of at least 0.005 mg/ml.

[1] Identified in a test solution, based on the Rf values provided by the manufacturer.
[2] This pot from the solution of 60 mg lumefantrine in 2 ml of ethyl acetate also illustrates that the solvent used in the method is selective for artemether and its related substances.

D.3 ROBUSTNESS

The spots of the aged samples were comparable to that of the freshly prepared samples. There was no significant difference between the Rf values (Table 4) of the initial and the aged sample, also with respect to the intensity and appearance. The test and standard solutions should be used within 48 hours after preparation. No comment with respect to the stability of these solutions is needed in the Ph.Int. monograph of artemether/lumefantrine tablets, since a period of 24 hours is sufficiently long for conducting the test.

Table 4 Rf values of artemether and related substances obtained in the test sample and standards for robustness

Substance	**Freshly prepared sample**		**Aged sample (after 48 hours)**	
	mm	Rf	mm	Rf
Mobile phase	120		120	
Artemether in test solution	66	0.55	67	0.56
Artemether in standard solution	65	0.54	67	0.56
α-artemether in standard solution	48	0.40	49	0.41
Artenimol in standard solution	36	0.30	37	0.31

D.4 SYSTEM SUITABILITY

The chromatogram obtained with standard solution (3) showed three spots which were clearly separated, thus the test was valid according to the criteria (Figure 2).

PART E: CONCLUSION

A TLC method for the limit test of artemether related substances in artemether/lumefantrine tablets has been developed and validated.

The method proved to be specific and robust with respect to the known related substances and lumefantrine does not interfere in the test. The test and sample solutions are stable for at least 48 hours, protected from light. Related substances present can be detected to a limit of 0.1%, with respect to artemether. In order to ensure sufficient separation between the spots a system suitability criterion was included in the method stating that standard solution (3) should produce three visible spots.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 5.

APPENDIX I

Validation of a high performance liquid chromatographic method for the assay of artemether and lumefantrine in artemether/lumefantrine tablets

PART A: OBJECTIVE

The objective is to validate the high performance liquid chromatographic method for assay of artemether and lumefantrine in artemether/lumefantrine tablets. In order to evaluate and validate this method according to the ICH Guidelines on Validation, ICH Q2(R1) (2005:3), accuracy, precision (repeatability and reproducibility), specificity, linearity and range and robustness of this method will be investigated.

The tablets normally contain:

Lumefantrine	120 mg (108 – 132 mg)
Artemether	20 mg (18 – 22 mg)

PART B: HPLC ASSAY TEST PROCEDURES

B.1 ORIGIN OF METHOD

Method from manufacturer supplied by the WHO.

B.2 PRINCIPLE OF METHOD

To simultaneously determine the content of artemether and lumefantrine APIs in the artemether/lumefantrine tablets by means of HPLC.

B.3 EQUIPMENT

- HP1100 series HPLC equipped with a HP1100 quaternary gradient pump, HP1100 auto sampler, HP 1100 diode array detector and Chemstation Rev. A.09.03 (1417) data acquisition and analysis software (Agilent, Germany)
- Column: Symmetry C_{18} 5 µm, 100 Å (Waters) 150 mm x 3.9 mm (or equivalent)
- Glassware (Grade A, class 1)
- Millipore filters: 0.45 µm hydrophilic PVDF membrane (Microsep, South Africa).

B.4 REAGENTS, SAMPLES, SOLVENTS AND SOLUTIONS

- Artemether primary reference standard (RS), provided by the manufacturer (Table 1)
- Lumefantrine primary reference standard (RS), provided by the manufacturer (Table 1)
- Artemether/lumefantrine tablets (Table 1)
- Acetonitrile R (Merck, HPLC grade)
- 1-Propanol R (Merck, AR grade)
- Phosphoric acid R (Merck, AR grade)
- Water R (MilliQ)
- Sodium hexanesulfonate R (Merck, AR grade)
- Sodium dihydrogen phosphate R (Merck, AR grade)
- Ion pair reagent: Dissolve 5.65 g of sodium hexanesulfonate and 2.75 g of sodium dihydrogen phosphate in about 900 ml of water. Adjust pH to 2.3 using phosphoric acid. Dilute to 1000 ml and filter (0.45 µm).
- Mobile phase A: Ion pair reagent : acetonitrile (700 : 300)
- Mobile phase B: Ion pair reagent : acetonitrile (300 : 700)
- Solvent: Mix 200 ml of ion pair reagent, 60 ml of water and 200 ml of 1-propanol and dilute to 1000 ml with acetonitrile.

Table 1 Primary reference standards and test sample used for validation of HPLC assay method

Description	Batch number	Manufacturing Company
Lumefantrine RS	C0189	Novartis
Artemether RS	C0015	Novartis
Artemether/lumefantrine 20/120 mg tablets	X1435	Novartis (Coartem® 20/120)

B.5 TEST SOLUTION

Weigh and powder 20 artemether/lumefantrine 20/120 mg tablets. To a quantity of the powder containing about 20 mg of artemether (120 mg lumefantrine), accurately weighed, add 85 ml of solvent, sonicate for 20 minutes and allow to cool to room temperature. Dilute to 100 ml with solvent. Filter through a 0.45 µm filter, discarding the first few ml of the filtrate. This is solution (1).

B.6 STANDARD SOLUTION

For solution (2) accurately weigh 20 mg of artemether RS and 120 mg of lumefantrine RS in a 100 ml volumetric flask. Add approximately 85 ml of solvent, sonicate until dissolved, allow to cool to room temperature and dilute to volume with solvent.

B.7 CHROMATOGRAPHIC CONDITIONS AND MEASUREMENT PROCEDURE

Table 2 Gradient table for the assay test of artemether and lumefantrine

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Comments	Detection wavelength (nm)
0 – 28	60	40	Isocratic	210
28 – 29	60 to 0	40 to 100	Linear gradient	380
29 – 45	0	100	Isocratic	380
45 – 46	0 to 60	100 to 40	Return to initial composition	380
46 – 55	60	40	Isocratic re-equilibration	380

Flow rate: 1.3 ml/min (pressure about 150 bar).

Injection volume: 20 µl, equivalent to about 4 µg of artemether and about 24 µg of lumefantrine in the test solutions and the reference solution.

Detection: UV 210 nm for the first 28 minutes and then switch to 380 nm.

Temperature: Ambient (22 – 25°C).

Retention times: artemether: about 19 minutes, lumefantrine: about 34 minutes.

Inject standard solutions, and measure the areas of the peak responses for system suitability. Inject test solutions and measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of artemether and lumefantrine.

B.8 INTERPRETATION AND CALCULATION OF RESULTS

From the data obtained as discussed in section B.7 calculate the content of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) in the tablets using the following calculation:

$$\text{mg/tablet} = \frac{A_2 \times \text{mg STD} \times 100 \times \text{mg 20 tabs} \times \% \text{ Pot STD}}{A_1 \times 100 \times \text{mg SA} \times 20 \times 100}$$

Where: A_1 = Area standard solution

A_2 = Area sample solution

mg STD = mass of reference standard weighed for standard solution

mg SA = mass of powdered sample weight for analysis

mg 20 tabs = mass of 20 tablets powdered for analysis

% Pot STD = % potency of reference standard used

PART C: VALIDATION PARAMETERS, TEST PROCEDURES AND ACCEPTANCE CRITERIA

The ICH guideline (ICH Q2(R1), 2005:1-13) will serve as reference for the validation of this analytical method.

C.1 SPECIFICITY

- Prepare a standard solution (2) (according to section B.6) and an analytical placebo[1] [weigh an amount equivalent to that of one tablet and prepare the sample using the procedure for test solutions (section B.5)].
- Carry out the measurement procedure (B.7) on the standard solution (2) and the analytical placebo.
- Examine the chromatograms for any additional peaks that may interfere with those of the active ingredients.
- Prepare a standard solution as described in section B.6. From this standard solution prepare four individual solutions diluting 10 ml of the standard with 5 ml of each of the following 0.1 N HCl (acidic), 0.1 N NaOH (alkaline), 3% H_2O_2 (oxidative) and water to create stress media. Follow the measurement procedure as discussed in section B.7 over a period of 24 hours.

Acceptance criterion:

- The placebo sample should show no peaks interfering with those of the APIs (artemether and lumefantrine).
- Extra peaks formed under stress conditions should be discernable from those of the active ingredients.

C.2 LINEARITY AND RANGE

- Prepare solutions (following the procedure in B.6) containing 80, 90, 100, 110 and 120% of the label claim (20 mg artemether and 120 mg lumefantrine).
- Carry out the measurement procedure (B.7) on these solutions.
- Conduct a linear regression analysis.

[1] The analytical placebo consists of the following: polysorbate 80 (100 mg), hypromellose (100 mg), microcrystalline cellulose (500 mg), colloidal anhydrous silica (50 mg), croscarmellose sodium (100 mg) and magnesium stearate (60 mg).

Acceptance criterion:

- The method should be linear from 80 – 120% and the R^2 between the five samples should be ≥ 0.99.

C.3 ACCURACY

- Weigh an amount of the analytical placebo equivalent to that of one tablet as well as 20 mg of artemether RS (accurately weighed) and 120 mg of lumefantrine RS (accurately weighed) and transfer to a 100 ml volumetric flask. Add approximately 85 ml of solvent, sonicate for 20 minutes and allow to cool to room temperature. Dilute to volume with solvent and filter. This procedure was done in sixfold.
- Carry out the measurement procedure (described in section B.7) on all six these solutions.
- Calculate the mean recovery and the %RSD.

Acceptance criteria:

- The difference between the % mean recovery and the actual (100%) must be ± 2.0% (Recovery should be 98.0 – 102.0%).

C.4 PRECISION

C.4.1 Repeatability

- Use the data obtained for accuracy.
- Calculate the mean recovery and the %RSD to determine the repeatability.

Acceptance criterion:

- The %RSD calculated from the mean recovery of the six samples should be ≤ 2.0% for both APIs.

C.4.2 Reproducibility

- On different days two different analysts should carry out the measurement procedure (section B.7) on the same batch of artemether/lumefantrine tablets in different laboratories.
- Calculate the mean recovery and %RSD.

Acceptance criteria:

- The %RSD obtained by each analyst should be ≤ 2.0% for each API, and the %RSD for each API between all the samples (tested by the different analysts) should be ≤ 3.0%.

C.5 ROBUSTNESS

- Prepare the mobile phase with ion pair reagent pH 2.1 and carry out the measurement procedure (as described in section B.7).

- Prepare the mobile phase with ion pair reagent pH 2.5 and carry out the measurement procedure (as described in section B.7).
- Carry out the measurement procedure (as described in section B.7) using a similar column from a different manufacturer.
- Store a solution (100% of target concentration) prepared for accuracy testing at ambient conditions for 48 hours and carry out the measurement procedure (as described in section B.7) for the aged solution on the same system.
- Examine the chromatograms for changes in retention time and peak area and note changes that influence the chromatography.

Acceptance criteria:

- Poor chromatography due to any of these changes should lead to a note in the method.
- The aged solution should not differ from the initial solution with more than 2.0%.

PART D: SUMMARY AND DISCUSSION OF VALIDATION RESULTS

Table 3 Summary of validation results for HPLC assay method

Parameter	Acceptance criteria	Results
Specificity	The solvent and placebo solution should not generate any peaks that will interfere with the determination of the active ingredients (artemether and lumefantrine), any other peaks should be discernable from those of these two actives. For stressed samples no interference of the API peak should be detected.	Absence of interference was demonstrated for the solvent and the placebo solution (Figure 1, 2, 3 and 4). No interferences with the API peaks were detected after stress testing (peak purity measurement Figure 5 and 6).
Linearity & range	The method should be linear over a range of 80 – 120% with the correlation coefficient (R^2): ≥ 0.99.	The method proved to be linear for a concentration range of 80 – 120%. The R^2 for artemether was 0.9979 and for lumefantrine 0.9981. (Table 4, Figure 7 and 8).
Accuracy	The difference between the percentage mean recovery and the theoretical (100%) must be ± 2.0% (Recovery should be 98.0 – 102.0%).	The mean recovery for artemether: 100.9% (100.5 – 101.3%, Table 5). The mean recovery for lumefantrine: 100.1% (99.7 – 100.7%, Table 5).
Precision		
A: Repeatability (spiked solutions)	Repeatability of analysis on six solutions at 100% of the target concentration (200 µg/ml artemether and 1200 µg/ml lumefantrine). %RSD ≤ 2.0%.	The mean for artemether: 100.9% and %RSD = 0.3%. The mean for lumefantrine: 100.1% and %RSD = 0.4%.
B: Reproducibility (tablets)	The %RSD obtained by each analyst should be ≤ 2.0%. The %RSD of the six preparations between different analysts must be ≤ 3.0%.	Mean (%RSD) artemether Analyst A: 95.7% (0.5%), Analyst B: 95.7% (1.8%). Mean (%RSD) lumefantrine Analyst A: 99.8% (0.5%), Analyst B: 96.8% (1.3%). %RSD for artemether = 1.2%. %RSD for lumefantrine = 1.9%.
Robustness	Poor chromatography due to a change in pH should lead to a note in the method. The peak areas of the aged solution should not differ from the initial solution with more than 2.0%.	The change in the pH value of the ion pair reagent in the mobile phase had an insignificant effect on the retention time. The peak areas of artemether differed with 2.0% from the initial in 24 hours and with 2.3% in 48 hours. The peak areas of lumefantrine differed with 0.5% from the initial in 48 hours.

D.1 SPECIFICITY

The solvent and placebo chromatograms (Figure 1 and 2) showed no peaks interfering with those of the APIs (artemether and lumefantrine) in the standard and sample chromatograms (Figure 3 and 4). The method proved to be specific with regards to both APIs.

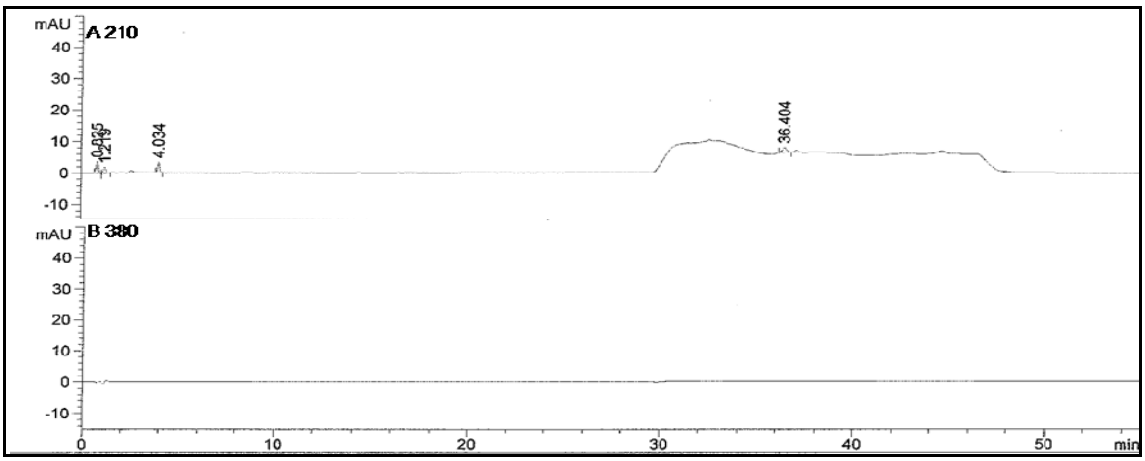


Figure 1 Solvent chromatograms for artemether/lumefantrine tablets.

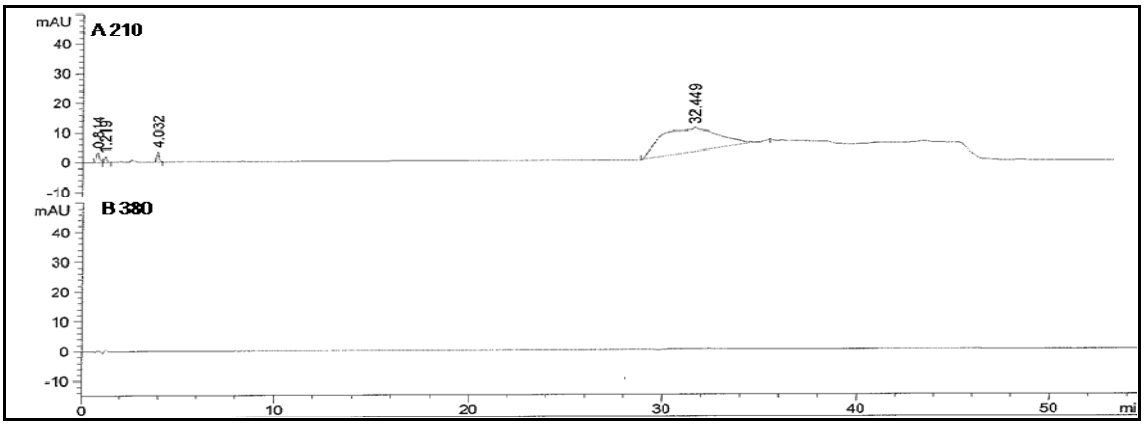


Figure 2 Analytical placebo chromatograms for artemether/lumefantrine tablets.

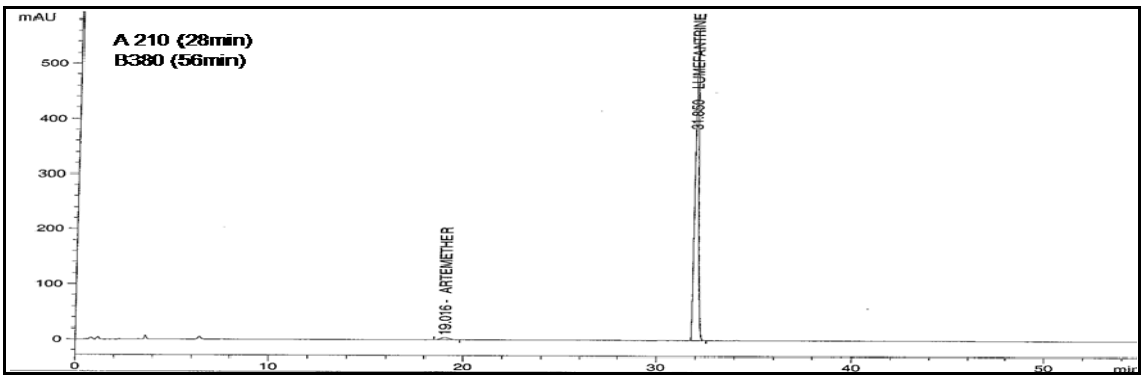


Figure 3 HPLC chromatogram of standard solution for artemether/lumefantrine tablets assay.

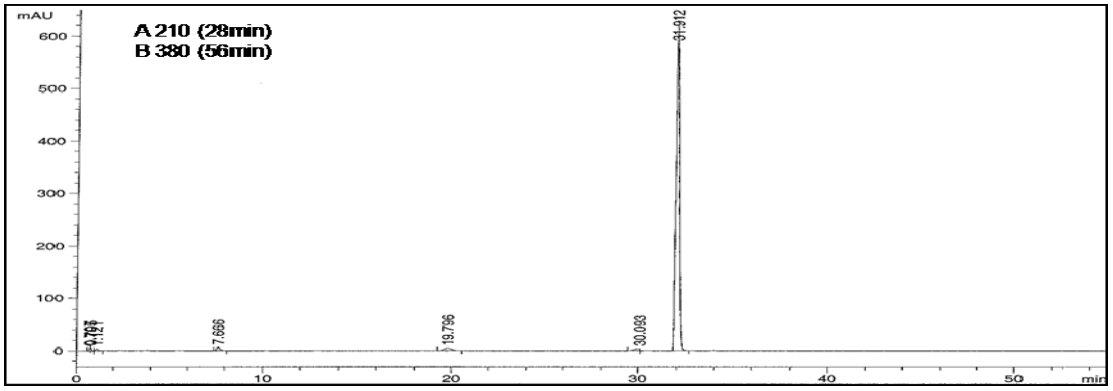


Figure 4 HPLC chromatogram of test solution for artemether/lumefantrine tablets assay.

The purity of the artemether and lumefantrine peaks were evaluated by means of diode array detection, see Figure 5 and 6 and showed no difference in purity of more than 2.0% for any peak within 24 hours. This indicated that that the method is specific for artemether at a wavelength of 210 nm and for lumefantrine at a wavelength of 380 nm.

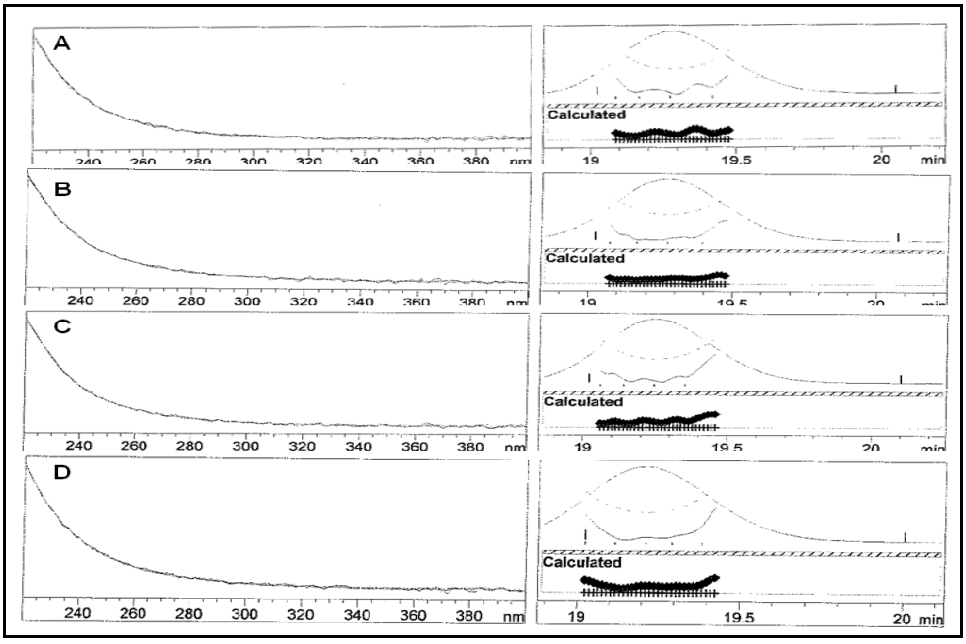


Figure 5 The peak purity profile of artemether in A: 0.1 N HCl, B: 0.1 N NaOH, C: 3% H_2O_2 and D: water.

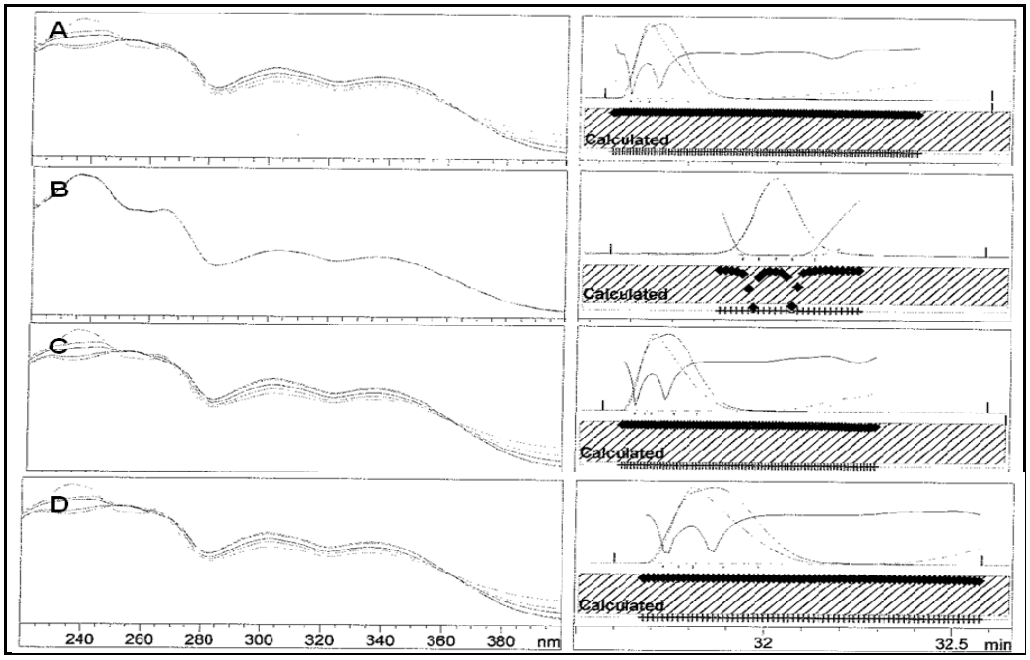


Figure 6 The peak purity profile of lumefantrine in A: 0.1 N HCl, B: 0.1 N NaOH, C: 3% H_2O_2 and D: water.

D.2 LINEARITY AND RANGE

The method proved to be linear over a concentration range of 80 - 120% of the expected sample concentration for both artemether (Table 4 and Figure 7) and lumefantrine (Table 4 and Figure 8). The regression coefficient for artemether is 0.9979 and for lumefantrine 0.9981, thus within the acceptance criterion of 0.99.

Table 4 The standard concentrations used and peak areas measured for artemether and lumefantrine linearity and range tests

Sample (%)	Artemether			Lumefantrine		
	Mass (mg)	Concentration (µg/ml)	Peak area	Mass (mg)	Concentration (µg/ml)	Peak area
80	16.30	163.0	115.6	96.04	960.4	6827.7
90	18.18	181.8	127.7	108.01	1080.1	7165.2
100	20.05	200.5	142.1	120.14	1201.4	8512.6
110	22.15	221.5	159.2	132.03	1320.3	9467.2
120	24.06	240.6	170.9	144.17	1441.7	10168.3

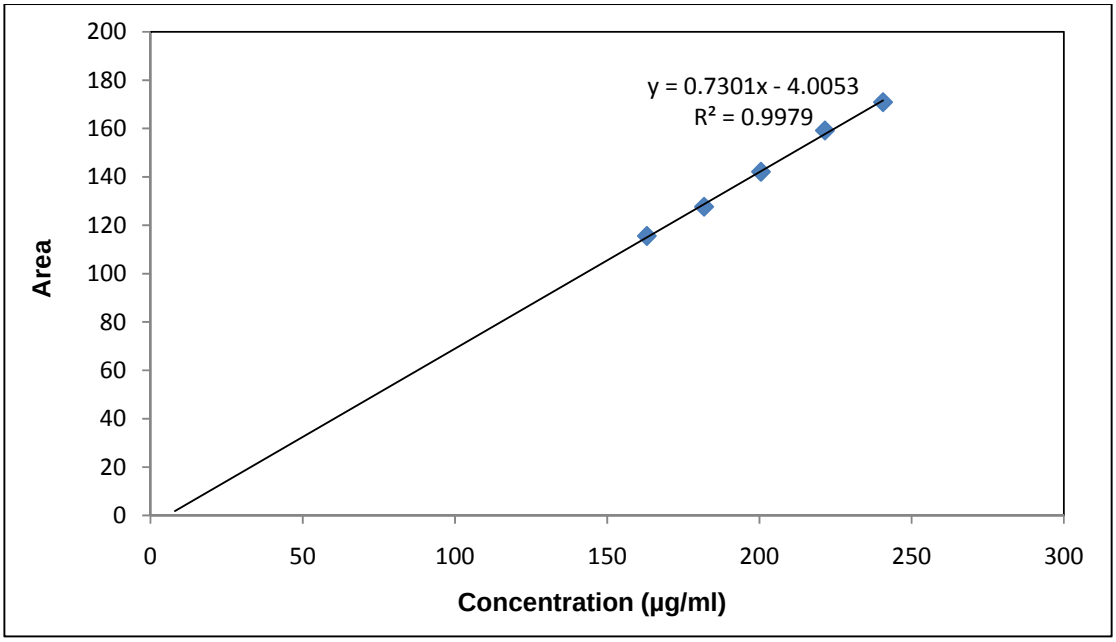


Figure 7 Linear regression graph for artemether.

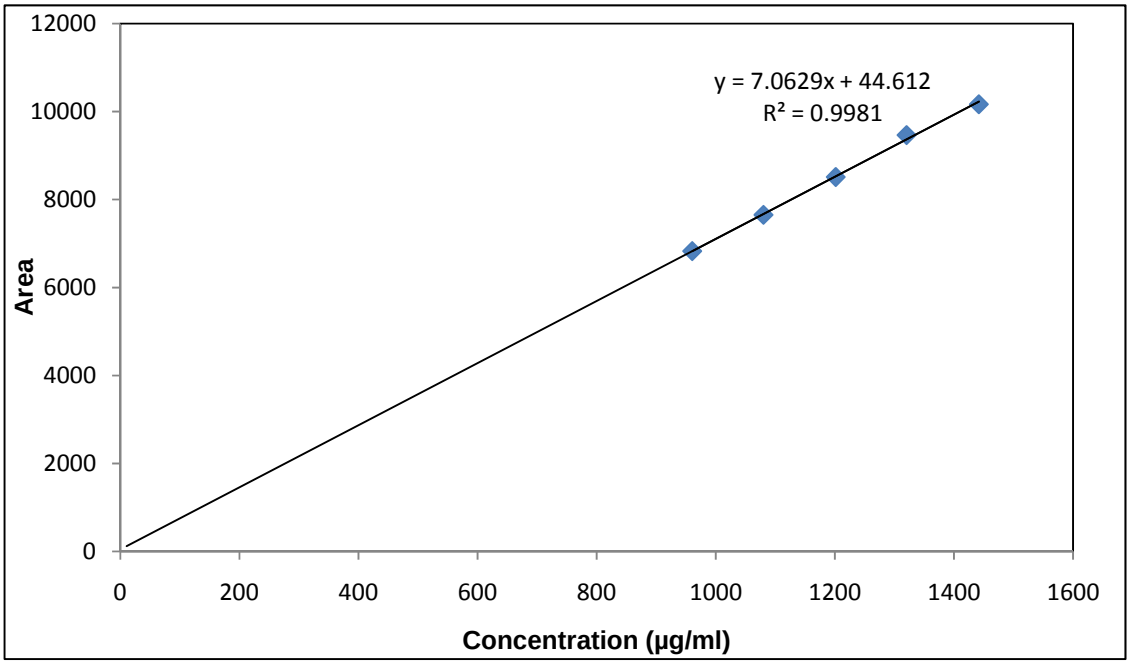


Figure 8 Linear regression graph for lumefantrine.

D.3 ACCURACY

In all six cases for the placebo spiked with a known quantity of the APIs the mean recovery were within the limits of 98 – 102% (Table 5) with 100.45 – 101.30% for artemether and 99.70 – 100.65% for lumefantrine.

Table 5 Recovery (%) of artemether and lumefantrine for accuracy and repeatability tests

Sample	Artemether			Lumefantrine		
	Mass (mg)	Area	% Recovery	Mass (mg)	Area	% Recovery
1	20.05	142.1295	100.45	120.41	8506.572	99.71
2	20.35	144.947	100.93	120.12	8521.953	100.13
3	20.30	144.8631	101.12	120.32	8499.845	99.70
4	20.20	143.7981	100.88	120.14	8512.605	100.00
5	20.17	144.1902	101.30	120.18	8565.598	100.59
6	20.10	142.5208	100.48	120.20	8571.841	100.65
Mean	**-**		**100.86**	**-**		**100.13**
%RSD	**-**		**0.3%**	**-**		**0.4%**

D.4 PRECISION

D.4.1 Repeatability

The repeatability results for artemether and lumefantrine are presented in Table 5. The %RSD of the six preparations at 100% target strength was 0.3% for artemether and 0.4% for lumefantrine.

D.4.2 Reproducibility

The data for reproducibility testing are presented in Table 6. Samples A1 – A3 were tested by analyst A on day 1 (%RSD was 0.5% for artemether and 0.5% for lumefantrine) and samples B1 – B3 were tested by analyst B on day 2 in a different laboratory (%RSD was 1.8% for artemether and 1.3% for lumefantrine). The %RSD between all samples was 1.2% for artemether and 1.9% for lumefantrine, thus within acceptance criteria limits (%RSD ≤ 3.0%).

Table 6 The assay of one batch of artemether/lumefantrine tablets by two analysts in different laboratories for reproducibility test

Sample (laboratory A[1])	% Assay artemether	% Assay lumefantrine	Sample (laboratory B[2])	% Assay artemether	% Assay lumefantrine
A1	95.5	99.2	**B1**	96.3	97.4
A2	96.2	99.8	**B2**	94.1	95.4
A3	95.3	100.4	**B3**	97.4	97.6
Mean	95.7	99.8	**Mean**	95.9	96.8
%RSD	0.5	0.5	**%RSD**	1.8	1.3

D.5 ROBUSTNESS

A change in the pH difference of the mobile phase did not influence the chromatography and assay values significantly, the assay stayed within 2%. The retention time of the standard and sample solutions increased slightly (with not more than one minute) for both APIs with a decrease in pH while both APIs eluted earlier (about one minute) with an increase in pH.

During the robustness testing a Luna C18 column 150 mm x 4.6 mm, 5 µm (Phenomenex) was used as a second column. The peak for artemether shifted from 20 minutes to 27.8 minutes, which could be expected considering the diameter of the column (4.6 mm instead of the prescribed 3.9 mm) while lumefantrine stayed at 32 minutes (Kazakevich & Lobrutto, 2007:35). It would be advised that the column used, first be tested with the gradient in order to change the gradient table if needed.

An injection volume of 20 µl was found to be sufficient and used as injection volume during the validation process as well as in the monograph.

The peak areas for artemether and lumefantrine did not differ from the initial with more than 2.0% (Table 7) within 48 hours.

[1] Laboratory A = Research Institute for Industrial Pharmacy.
[2] Laboratory B = Centre for Quality Assurance of Medicine.

Table 7 Percentage difference between peak areas of artemether and lumefantrine in 48 hours

Time (hours)	Peak area for artemether	Peak area for lumefantrine
0	129.5	7647
6	129.8	7651
12	131.2	7629
24	132.1	7658
48	132.5	7688

PART E: CONCLUSION

A HPLC method to simultaneously determine the assay of artemether and lumefantrine in artemether/lumefantrine tablets has been developed and validated.

The method proved to be specific for both APIs. Linearity was established over a range of 80 – 120%. Accuracy was demonstrated, while precision testing proved the method to be repeatable and reproducible (in different laboratories). The method is robust with respect to pH of ion pair reagent (mobile phase). The column should however be tested for efficacy before use and a specific column was advised in the method for the monograph.

PART F: BIBLIOGRAPHY

See Bibliography: Chapter 5.