

Chapter 1: The importance of quality anti-malarial treatment

Introduction

Malaria is endemic to 106 countries around the world (Figure 1-1, Figure 1-2) and is considered a global health priority of the World Health Organization (WHO) (Barnes, 2012:1 - 2). It is estimated that more than 200 million people are infected with the disease annually, resulting in approximately 655 000 deaths (WHO, 2011:xii). One of the major setbacks in the global fight against malaria is the use of counterfeit and/or substandard anti-malarial medication. The surveillance work of Bate *et al.* (2008:2133) and Gaudiano *et al.* (2007:1) serve as classic examples exemplifying the setback. From their collective works it was found that up to 50% of the surveillance samples (from various regions in Africa) did not conform to the quality standards which are to be expected from all medicines.

This study aims to contribute in the fight against malaria by critically investigating the analytical quality control test procedures and specifications of quinine sulfate tablets set by three international pharmacopoeial monographs.

The purpose of this chapter is to i) provide the reader with brief, yet fundamental background information on malaria, and ii) introduce the main attributes contributing to the safety and efficacy of medicines.

1.1 Introduction to malaria

1.1.1 Prevalence and transmission

Malaria is dependent on the prevalence of the vector (carrier), the anopheline mosquito, which is responsible for the transmission and distribution of *Plasmodium* (*P*) protozoa. There are four main types of *Plasmodium* protozoa; namely; *P. Falciparum*, *P. Vivax*, *P. Ovale* and *P. Malariae*. Infection due to *P. Falciparum* and *P. Vivax* are the most common, whereas infection by *P. Falciparum* is the deadliest of all the malaria parasites (Rang *et al.*, 2003:673).

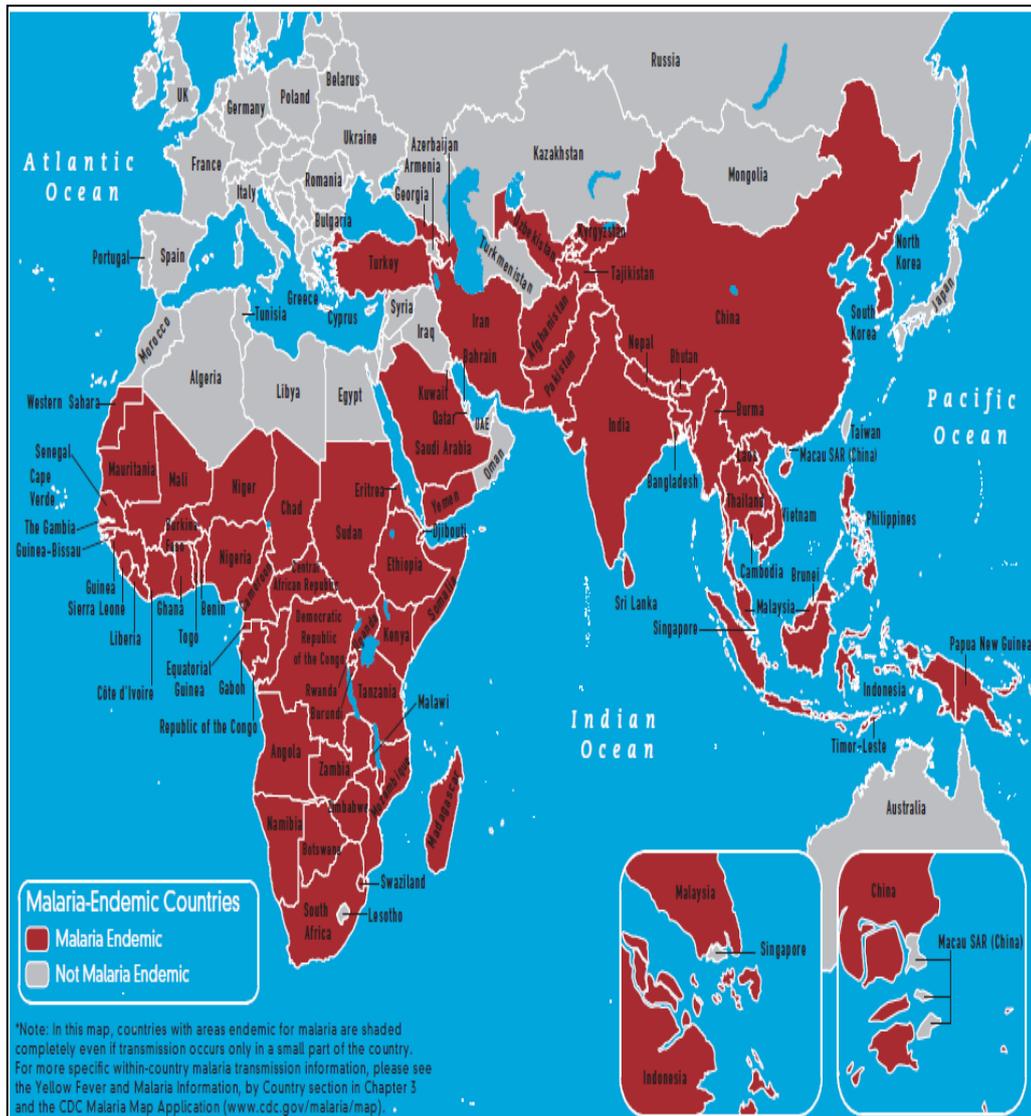


Figure 1-1: Malaria-endemic countries in the Eastern Hemisphere (Arguin & Mali, 2012).

Anopheline mosquitoes breed in water, resulting in higher occurrences and risk during (or shortly after) rainy seasons. Malaria is distributed in tropical areas, throughout sub-Saharan Africa, South East Asia, the Pacific Islands, India and Central and South America (Figure 1-1 and Figure 1-2). *P. Falciparum* is prevalent in most of these endemic countries (Ashley *et al.*, 2006:160).



Figure 1-2: Malaria-endemic countries in the Western Hemisphere (Arguin & Mali, 2012).

1.1.2 Parasitology

Infected anopheline mosquitoes transfer *Plasmodium* parasites (sporozoites) to the human host upon feeding (Rang *et al.*, 2003:673). The sporozoites that were deposited by the mosquito are then transported to the liver via the host's bloodstream, where tissue merozoites form within 10 - 14 days. These merozoites mature and cause the liver parenchymal cells to rupture, releasing the merozoites into the host's bloodstream. These merozoites enter erythrocytes and transform into intracellular parasites called trophozoites. The trophozoites proliferate within the erythrocytes and cause these cells to rupture. Subsequently even more merozoites are

released into the bloodstream which either infect new erythrocytes or transfer to another mosquito when feeding on the host (Rang *et al.*, 2003:673). The sexual phase of the parasite's life cycle continues within the mosquito when gametocytes are ingested by the mosquito (Barnes, 2012:3). An illustration depicting the life cycle is portrayed in Figure1-3.

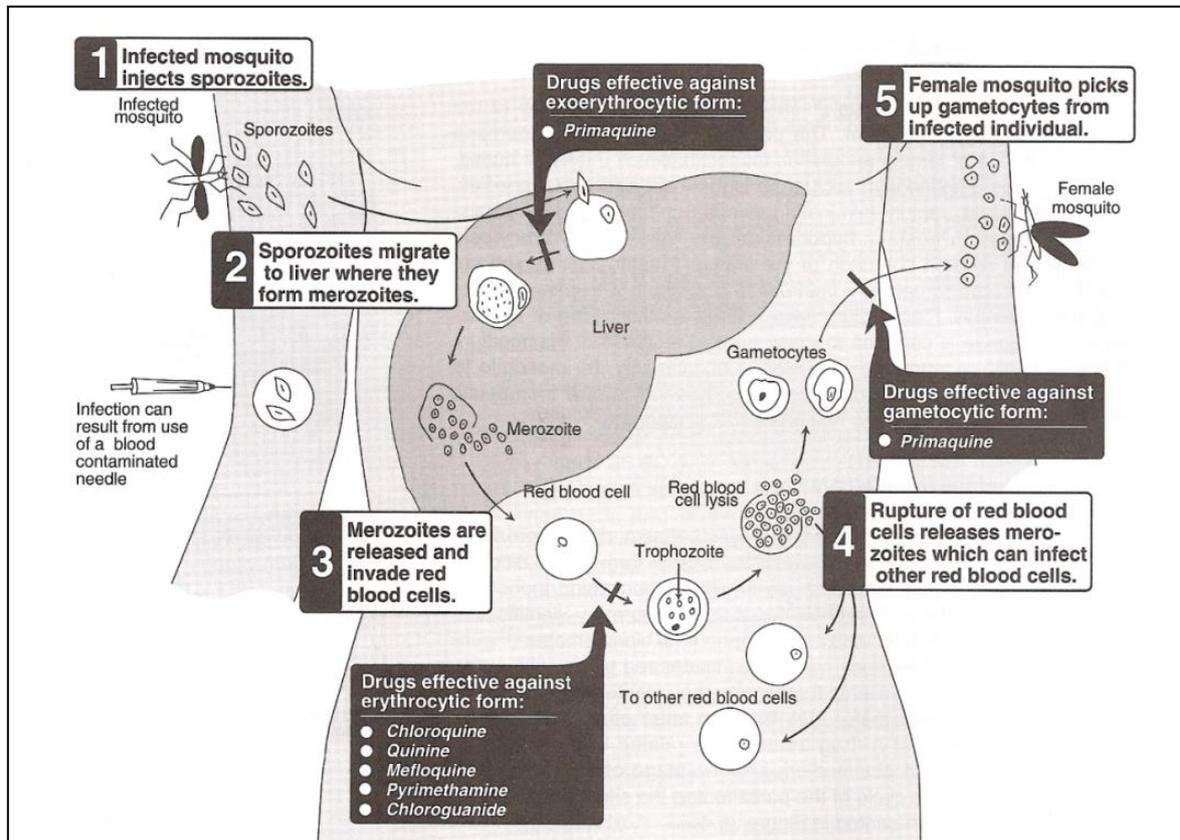


Figure 1-3: The life cycle of the malaria parasite showing target areas for anti-malarial treatment action (Harvey *et al.*, 2000:350).

1.1.3 Pathophysiology and clinical manifestations of malaria

The rupturing of the host's erythrocytes is the major cause of the clinical signs and symptoms of malaria (Barnes, 2012:3). The debris from the dead erythrocytes coagulates within the microcirculation of the host's blood system which causes hypoxia (Walker *et al.*, 2009:41). The most common symptoms of an uncomplicated malaria infection are high fever, chills, headaches, myalgia and gastrointestinal symptoms (Walker *et al.*, 2009:41; Ashley *et al.*, 2006:163). When not treated promptly, uncomplicated malaria will progress into severe complicated malaria which will cause hypoglycemia, metabolic acidosis, fluid and electrolyte disturbances, acute renal failure, acute pulmonary oedema, shock, septicaemia and generalised

convulsions. When the malarial infection is allowed to progress even further, the host may fall into a coma, which is known as cerebral malaria (Ashley *et al.*, 2006:164).

1.1.4 Diagnosis of malaria

The symptoms associated with malaria are similar to several other diseases and therefore diagnosis on these grounds may be unreliable and may result in unjustified treatment (Bell & Perkins, 2012:293). To ensure a correct diagnosis, the WHO recommends that a blood sample be tested for the presence of the malaria parasite. Only if the test is positive should anti-malarial treatment be prescribed (WHO, 2011:x).

1.1.5 Prevention and treatment

One means to prevent infection is by reducing the contact between the host and the carrier. For this reason insecticide-treated bed nets and a pyrethroid containing insect spray may be used in sleeping areas (Ashley *et al.*, 2006:161). Long sleeved clothing can also be worn (Arguin & Mali, 2012).

Prophylactic treatment (chloroquine, mefloquine, proguanil, pyrimethamine, dapsone and doxycycline) may be used by persons travelling to endemic regions (Rang *et al.*, 2003:676) as a preventative measure together with that mentioned above.

Malaria is treated with a variety of anti-malarial medicines. These anti-malarial medicines are classified according to their respective targets and mechanism of actions (Rang *et al.*, 2003:676):

- Primaquine is an anti-malarial agent that is classified as a schizonticidal agent, as it is effective against the proliferation of the parasites in the liver.
- Chloroquine, quinine, mefloquine, pyrimethamine and chloroguanide are classified as blood schizonticidal agents as they interrupt the life cycle by destroying the parasite within the erythrocytes of the host.

Of specific interest to this study is quinine (in the form of quinine sulfate), which will be discussed in more detail in Chapter 2.

1.2 The impact of - and means to combat poor quality medicine

It is estimated that 15 - 50% of the world's medicine are counterfeit and/or substandard (Amin & Kokwaro, 2009:430). Substandard- and counterfeit medicine continue to impair the effective treatment of malaria (Amin & Kokwaro, 2009:430; Vestergaard & Ringwald, 2007:153). The

terms “substandard” and “counterfeit” are closely related and therefore it is important to note their difference in meaning (Table 1-1).

Table 1-1: The difference between counterfeit and substandard medicines (adapted from Obi-Eyisi & Wertheimer, 2012:2 and WHO, 2003)

Criterion	Counterfeit medicine	Substandard medicine
Composition and ingredients do not meet the respective specifications	Yes	Yes
Deliberately and fraudulently mislabeled with respect to identity and/or source	Yes	No
Result of insufficient human and/or financial resources and/or lack of knowledge	Yes	Yes

The use of substandard anti-malarial medicine may lead to the malaria parasite building up resistance against these medications, rendering them ineffective for future use (Barnes, 2012:1-2). To successfully combat and eradicate malaria, medicine intended for therapy should be safe and effective.

The WHO defines quality control of medicines as “all the measures taken to ensure that finished/final pharmaceutical products (FPP) conform to established specifications for identity, strength, purity and other characteristics” (WHO, 2010). Section 1.3 elaborate on the different elements of quality control of medicines.

1.3 Medicine regulations

Medicine regulatory authorities (MRA's) inspect pharmaceutical manufacturing companies to ensure that Good Manufacturing Practice (GMP) is complied to and that the appropriate final Quality Control (QC) tests are performed on medicinal products prior to release. This ensures that medicine that reach a patient has been appropriately manufactured, stored, distributed and dispensed and are of good quality (WHO, 2013).

From the estimated annual malaria death toll, 91% were found to be in Sub-Sahara Africa (WHO, 2011). 31% of these deaths are reported to be unjustified seemingly due to ineffective MRA's in the African regions (AFM, 2009:4). It is estimated that 90% of these MRA's are unable (due to insufficient resources and guidance) to guarantee the quality, efficacy and safety of the medicines in their countries (Burki, 2010:222).

1.3.1 Good Manufacturing Practice (GMP)

Each step of the pharmaceutical manufacturing process should be controlled to ensure the quality of FPP (Anisfeld, 2012). GMP is defined as acceptable and applicable standards which guide pharmaceutical companies to consistently produce and control the quality of their products (WHO, 2012).

GMP guidelines comprise stringent recommendations on the management and control of the following (Brhlikova *et al.*, 2007:8):

- quality management systems,
- production facilities,
- records,
- in-process control checking,
- packaging,
- storage,
- distribution,
- laboratory controls,
- validation and
- complaints and recalls of medicines.

If a company fails to comply with GMP, the quality, safety and efficacy of the medicines/FPP are considered to be compromised (Anisfeld, 2012). Analytical QC testing forms part of GMP and is intended to ensure that a final product meets its quality specifications at the time of release to the market and throughout the shelf-life thereof (Anisfeld, 2012).

1.3.2 Analytical quality control (QC) testing

Analytical QC testing is the final confirmation by means of appropriate analytical procedures that the pharmaceutical product is of acceptable quality (Lee and Webb, 2003:3). Analytical test methods are specifically developed and validated for their respective purposes and are accompanied by justified specifications. Official international test methods and their

specifications provide international guidance to ensure safe and efficacious medicines (Ahuja, 2001:15).

Methods and specifications that are used to evaluate the quality of medicines should be developed in such a manner that the test conditions are fair, yet discriminatory (Shargel *et al.*, 2005:431). Employing specifications that are too lenient may contribute to a false belief into the quality of a medicine, and may result in substandard medicines being used for treatment. On the other hand, methods and specifications that are too stringent may allow for the rejection of medicines of acceptable quality (Shargel *et al.*, 2005:431). Great care should therefore be taken when developing methods and setting specifications (Shargel *et al.*, 2005:428).

Official test methods that used for testing are compiled in an official compendium, called a pharmacopoeia (WHO, 2003:24). The pharmacopoeia is published by a governmental authority or a medical pharmaceutical society (FDA, 2007). When a medicine complies with the specifications of the monograph, it is said to be of pharmacopoeial quality (WHO, 2003:24). The most commonly used pharmacopoeia according to the Food and Drug Administration (FDA, 2007) and Anisfeld (2012) are the:

- British Pharmacopoeia (BP);
- European Pharmacopoeia (Ph.Eur.);
- Japanese Pharmacopoeia (JP);
- United States Pharmacopeia (USP) and the
- International Pharmacopoeia (*Ph.Int.*).

The most common analytical tests (applicable to solid oral dosage forms) that are addressed by pharmacopoeia are described in sections 1.3.2.1 to 1.3.2.6 and summarised in Figure 1-4.

1.3.2.1 Identification

Identification tests confirm the presence of the active pharmaceutical ingredient(s) (API) in the FPP (Birrer *et al.*, 2001:272). Techniques commonly employed for identification include: infrared spectroscopy (IR), ultraviolet-visible (UV-Vis) spectroscopy, colorimetric methods, thin-layer chromatography (TLC), counter-ion precipitation reactions and high-performance liquid chromatography (HPLC) (USP, 2013).

1.3.2.2 Assay

The assay test is used to quantify the amount of the pharmaceutical moiety (or API) in the dosage unit using a pooled sample (usually at least 20 tablets). Results generated from such data are compared to the label claim of the product to verify that the correct amount of API is present in the sample (within certain acceptable limits, usually 90 - 110% of the label claim). Techniques that are used for assay testing include (but are not limited to): aqueous-, non-aqueous- and complexometric titration, UV-Vis spectroscopy and HPLC (USP, 2013; Richardson, 2001:333).

1.3.2.3 Uniformity of dosage units

Uniformity of dosage units ensure that there is a uniform dose of API in each dosage unit administered to the patient. Uniformity of dosage units is tested by weight variation, uniformity of mass or content uniformity (USP, 2013; BP, 2013). Uniformity of mass and weight variation evaluate the variation of the masses of individual dosage units from the average mass of the dosage units, whereas content uniformity assays a predetermined number of individual dosage forms (usually ten individual units). Results must be within specified limits of variance for the test to comply. Different monographs have different ways to perform and evaluate the results of these tests (weight variation/uniformity of weight), but in essence the same parameter (i.e. degree of variation) is tested for (Alderborn, 2007:461). Similar techniques used for assay testing is used for content uniformity testing.

1.3.2.4 Impurities or related substances

Three different types of impurities are known: organic impurities, inorganic impurities and residual solvents (although residual solvent testing only applies to the raw material).

Impurity testing foremost determines the presence and the concentration of these impurities in the sample. Sometimes assay testing and impurity testing follows the same HPLC procedures (Richardson, 2001:336). Other techniques include (but are not limited to): TLC, gas chromatography (GC) and atomic absorption spectrophotometry (AA). Compliance to the specifications set for the impurities ensure the product to be safe (non-toxic) for use.

1.3.2.5 Disintegration

For an API to be available for absorption it must be in solution. In order for this to happen, the solid oral dosage form containing the API must first disintegrate (Shargel *et al.*, 2005:414). A disintegration test determines whether a solid oral dosage form breaks-up into smaller particles

within a specified time when it is placed in a liquid medium for a predetermined amount of time (USP, 2013).

1.3.2.6 Dissolution

The process by which a solid dissolves in a medium as a function of time is defined as dissolution. Dissolution is an important pre-cursor for systemic absorption and thus bioavailability (Shargel *et al.*, 2005:414). The dissolution test will provide assurance that a solid oral dosage form will dissolve in the gastrointestinal fluids and through absorption and delivery, reach the target area via the bloodstream to provide the intended pharmaceutical effect/response (USP, 2013).

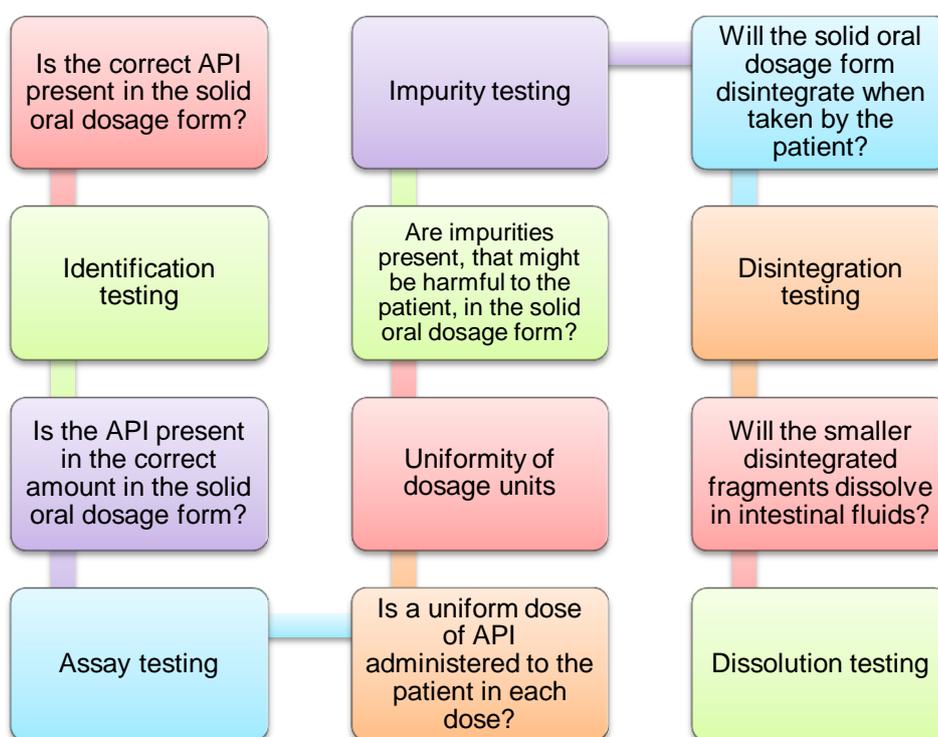


Figure 1-4: An overview and rationale of the pharmacopoeia tests that apply to solid oral dosage forms.

1.3.3 The suitability of QC analytical procedures and specifications

There is an increasing demand to verify the quality of medicine through the use of pharmacopoeial monographs, thus increasing the demand for well validated analytical methods that are fit for their purpose (Anisfeld, 2012). It is accepted that a validated method will

consistently provide accurate and reliable test results (Ahuja, 2001:4) (*Note: more detail on validation and the integrity of methods follow in Chapter 3 and 4*).

Pharmacopoeial requirements for the same medicinal product and dosage form may differ from each other (see an example in Table 1-2). It is expected from different validated test methods/techniques that are employed for the same test and test product, not to necessarily produce exactly the same results, but to produce comparable outcomes. Institutions such as the Pharmacopoeial Discussion Group (PDG) aim to harmonise pharmacopoeia (USP, 2013) for this very reason. According to PDG “a pharmacopoeial general chapter or other pharmacopoeial document is harmonised when a pharmaceutical substance or product tested by the document's harmonised procedure yields the same accept/reject decision” (USP, 2013).

Referring to Table 1-2, it is understandable that the solubility of the API will differ in different dissolution media, therefore justifying differences in the pharmacopoeial specifications. This exemplifies that the same test product need not provide the same result in each scenario, but should provide the same final outcome (the same product should comply to different criteria under different test conditions).

Table 1-2: Summary of the differences in dissolution procedures and specifications pertaining to quinine sulfate tablets (adapted from quinine sulfate tablet monographs of the USP, BP and *Ph.Int.*)

	BP	USP	<i>Ph. Int.</i>
Apparatus	Basket	Basket	Paddle
Dissolution medium	0.1 M HCl	0.01 M HCl	Buffer pH 6.8
Agitation speed (rpm)	100	100	75
Withdrawal time	45 minutes	45 minutes	30 minutes
Specification	Not less than 70% (Q) in 45 minutes	Not less than 75% (Q) in 45 minutes	Not less than 80% in 30 minutes
Method of quantification	UV (330 nm)	UV (248 nm)	UV (348 nm)

Conclusion

Malaria is a world-wide health concern. Despite an arsenal of means to prevent and treat malaria, it still remains responsible for an obscenely high death toll. It is evident from the available literature that malaria endemic countries are plagued with poor treatment efficacy because of substandard medicines. Analytical QC test methods have the means to determine

the quality of medicines and when effectively employed, may aid in eradicating substandard medicines from the market.

As with many aspects of GMP, analytical QC procedures and specifications should be routinely revised and harmonised to ensure that they remain current and suitable. This study critically evaluates the quinine sulfate tablet monographs of the BP, USP and *Ph.Int.* A brief discussion on quinine sulfate is given in Chapter 2, to ensure adequate background on this API.