

COMMENTARIES

Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Amodiaquine Hydrochloride

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ABSTRACT: The present monograph reviews data relevant to applying the biowaiver procedure for the approval of immediate release (IR) multisource solid dosage forms containing amodiaquine hydrochloride (ADQ) as the single active pharmaceutical ingredient (API). Both biopharmaceutical and clinical data of ADQ were assessed. Solubility studies revealed that ADQ meets the “highly soluble” criteria according to World Health Organization (WHO) and European Medicines Agency (EMA) but fails to comply with the United States Food and Drug Administration (US FDA) specifications. Although metabolism hints at high permeability, available permeability data are too scanty to classify ADQ unequivocally as a Class I drug substance. According to WHO and EMA guidances, ADQ would be conservatively categorized as a Class III drug, whereas according to the US FDA specifications, it would fall into Class IV. ADQ has a wide therapeutic index. Furthermore, no cases of bioequivalent products have been reported in the open literature. As risks associated with biowaiving appear minimal and requirements for “highly soluble” API are met in the WHO and EMA jurisdictions, the biowaiver procedure can be recommended for bioequivalence (BE) testing of multisource IR products containing ADQ as the only API, provided the test product contains excipients used in ADQ products approved in International Conference of Harmonisation and associated countries, and in similar amounts. Furthermore, both comparator and test should conform to “very rapidly dissolving” product criteria ($\geq 85\%$ dissolution of the API in 15 min at pH 1.2, 4.5, and 6.8) and the labeling should specify that the product not be coadministered with high-fat meals. If the comparator and/or test product fails to meet these criteria, BE needs to be established by pharmacokinetic studies in humans. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association *J Pharm Sci* 101:4390–4401, 2012

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INTRODUCTION

The feasibility of using surrogate *in vitro* dissolution tests to evaluate bioequivalence (BE) (biowaiver procedure) of multisource amodiaquine hydrochloride (ADQ) solid oral dosage forms is reviewed in this monograph. The assessment is based on the clinical and biopharmaceutical data of ADQ obtained from open scientific literature and supported by experimental data generated by performing additional

solubility and dissolution studies. Furthermore, the risks (of an incorrect biowaiver decision and its probable consequences to the individual patient and/or public health) associated with using the biowaiver procedure to evaluate the BE of ADQ multisource and reformulated products are evaluated. This assessment refers to drug products containing ADQ as the only active pharmaceutical ingredient (API) and not to combination drug products.

The purpose and scope of the biowaiver monograph series have been previously discussed.¹ The prerequisites for a biowaiver and the approach to the risk benefit analysis for a given API are based on the guidelines put forth by the World Health Organization (WHO),² European Medicine Agency (EMA),³ and United States Food and Drug Administration (US FDA).⁴ Biowaiver monographs of several APIs belonging to various therapeutic classes have already been published. These monographs are also available on the International Pharmaceutical Federation website (URL: <http://www.fip.org/www/index.php?id=642>).

METHODS

Literature Research

Literature research was performed to collect relevant data pertaining to the API, dosage, indication, toxicity, therapeutic index, safety, solubility, permeability, pharmacokinetics (PK), bioavailability (BA), BE, bioequivalence, and excipients interaction of ADQ. Information was obtained from general pharmaceutical literature and PubMed Central.

Solubility Class Determination

Solubility studies on ADQ were performed in buffer solutions (pH 1–7.5) and water (pH 6.8) by a modified shake flask method, in triplicate. Three milliliters of medium was added to around 25 mg of the API preweighed into Uniprep[®] vials (Whatman, Inc, New Jersey, USA) and gently shaken in an orbital shaker for 48 h at $37 \pm 0.5^\circ\text{C}$. Drug content in each sample was determined by measuring its absorbance after suitable dilution at 342 nm against appropriate blanks using ultraviolet (UV) spectrophotometry (U-3000 Spectrometer; Hitachi Ltd., Tokyo, Japan). The drug concentrations were calculated from calibration curves of the reference API in the respective medium.

In Vitro Dissolution Study

Dissolution studies were performed in triplicate on pure ADQ (Lot no.: 038F0993, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) according to the WHO specifications. ADQ, equivalent to 153 mg of amodiaquine base, was accurately weighed into each empty gelatin capsule. Three standard dissolution media, namely simulated gastric fluid without pepsin at pH

1.2 (SGFsp), acetate buffer at pH 4.5, and simulated intestinal fluid without pancreatin at pH 6.8 (SIFsp) were used. The United States Pharmacopoeia (USP) apparatus 2 containing 900 mL medium maintained at $37 \pm 0.5^\circ\text{C}$ was used with a paddle rotation speed of 75 rpm. Five milliliter samples were withdrawn manually at 5, 10, 15, 20, 30, 45, and 60 min using glass syringes through stainless steel sampling tubes fitted with cylindrical polyethylene filter sticks and were again filtered through $0.45 \mu\text{m}$ Whatman filter units (Schleicher & Schuell GmbH, Dassel Germany). The withdrawn samples were replaced by fresh medium maintained at $37 \pm 0.5^\circ\text{C}$. The samples were suitably diluted with the dissolution medium and analyzed for the drug content using UV spectrophotometer by measuring absorbance against suitable blanks.

GENERAL CHARACTERISTICS

Name: Amodiaquine hydrochloride (BANM, rINNM).

4-(7-Chloro-4-quinolylamino)-2-(diethylaminomethyl) phenol dihydrochloride dihydrate.⁵

4-[(7-Chloro-4-quinolyl)amino]- α -(diethylamino)-o-cresol dihydrochloride dihydrate^{6,7}

The compound has a molecular weight of 464.8g/mol and a melting point of 158°C .^{5,8,9}

Therapeutic Indication and Dosage

Amodiaquine hydrochloride is a Mannich base 4-aminoquinoline antimalarial recommended in the treatment of uncomplicated malaria caused by *Plasmodium falciparum*.^{5,10} After absorption, it is metabolized chiefly to desethylamodiaquine (ADQm), which also shows antimalarial activity. The WHO recommends the use of ADQ together with artesunate for the treatment of uncomplicated *falciparum* malaria, to reduce the risk of drug resistance compared with monotherapy.¹⁰ Doses of ADQ are generally expressed in terms of its free base. Two hundred milligrams of ADQ is equivalent to 153 mg of amodiaquine base. The doses specified in this manuscript refer to the amount of base unless specified otherwise.

The Summary of Product Characteristics (SmPC) of ADQ tablets (150 mg; Guilin Pharmaceutical Company Ltd., Shanghai, China) recommends a total dose of 35 mg/kg of amodiaquine base administered

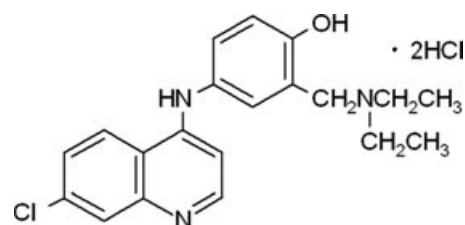


Figure 1. Structure of amodiaquine dihydrochloride.⁶

over the 3-day duration of therapy. On the first day, a dose of 15 mg/kg divided into two dosages of 7.5 mg/kg each is recommended, whereas on the second and third days, a dose of 10 mg/kg/day divided in two dosages of 5 mg/kg each is to be administered.¹¹ The WHO Malarial Treatment Guideline recommends a target dose of 10 mg/kg/day for 3 successive days with a therapeutic dose range of 7.5–15 mg/kg/day.¹⁰

Therapeutic Index and Toxicity

Amodiaquine hydrochloride can be considered as a broad therapeutic index API as the definition specified in the regulations at 21 CFR 320.33(c) of the US FDA for narrow therapeutic index drugs does not apply.¹² Additionally, although ADQ has been used for treatment of malaria for six decades, it did not appear in the list of narrow therapeutic range drugs published in the Scale-Up and Post-Approval Changes guidelines 1 in 1995.¹³ No data on the toxicity of ADQ after repeated oral administration to animals were available in the open literature. Single-dose toxicity studies reported a median lethal dose (LD₅₀) of 225 mg/kg (mouse intraperitoneal), LD₅₀ of 550 mg/kg (mouse oral), and maximum tolerable dose (LD₀) of 137 mg/kg (mouse intraperitoneal).^{11,14}

The adverse effects of ADQ are similar to those of chloroquine. Symptoms of an overdose include headache, dizziness, visual disorders, cardiovascular collapse, and convulsions followed by early respiratory and cardiac arrest.^{15–17} Serious, and in some cases fatal, hepatic and bone marrow toxicity has been observed in European travelers when used as a prophylactic.^{18–21} Agranulocytosis usually developed between 5 and 14 weeks of prophylaxis and was associated with hepatitis.²²

Retinopathy is rare; only a single case of corneal and conjunctival abnormalities has been noted. This adverse effect was observed in a 34-year-old man who ingested more than 250 g during 1 year of ADQ for pain treatment.²³ No neurological disorder has been reported, except for one case of muscle weakness, myalgia, corneal edema, and lethargy with muscle degeneration after 3 months of therapy in a 33-year-old white male taking 200 mg ADQ twice daily for photosensitive skin reaction.²⁴

In one study of ADQ, slight prolongation of PR, QRS, and QTc2 intervals on the electrocardiogram was observed in adult patients with acute uncomplicated *P. falciparum* infection. These variations were deemed clinically insignificant and were thought to be caused by the disease itself rather than the drug, as the effect did not correlate with the peak plasma concentration of amodiaquine or with that of ADQm, its chief metabolite.¹³

PHYSICOCHEMICAL PROPERTIES

Solubility

The United States Pharmacopoeia lists ADQ as water soluble at room temperature.⁶ Literature research revealed a solubility of one part in 22 parts of water for ADQ. However, the temperature at which the tests were performed was not reported.^{9,17} Additional Biopharmaceutics Classification System (BCS)-conform solubility studies were performed using Uniprep[®] vials in an adaptation of the shake flask method over the pH range of 1–7.5 at 37 ± 0.5°C in triplicate. The experiments were downsized and approximately 25 mg drug was weighed into 3 mL of aqueous buffer medium (this ratio corresponds to more than 2.6 times the highest recommended dose of 600 mg of amodiaquine base in 250 mL). In acidic media, the entire amount was dissolved; thus, the concentrations achieved in those media do not represent the saturation concentration. At higher pH (pH 6.8 and above), saturation was achieved and the values reported at these pH are solubility values. The conversion of the hydrochloride salt to the free base form of ADQ and the diprotic nature of ADQ free base contribute to the steep decline in the solubility at higher pH (6.8–7.5). Table 1 indicates the (maximum) dose–solubility ratios (D/S) of ADQ, according to the WHO, EMA, and US FDA specifications.

Simulated intestinal fluid without pancreatin at pH 6.8 at a higher buffer capacity (80 mM instead of 45 mM) was used to maintain the pH of the solution within the permissible limits throughout the testing. Precipitation of the free base was observed immediately after addition of ADQ in SIF_{sp} at pH 6.8, 7.0 and 7.5. For the highest single dose, D/S was not calculated at pH 7.0 and 7.5 because only the US FDA requires D/S to be assessed at these pH values and the US FDA uses only the highest dose strength as the basis for calculation of D/S.

Polymorphism and Partition Coefficient

No polymorphs of ADQ have been reported. Experimental studies on the partitioning of ADQ between 1-octanol and 0.1 M phosphate buffer and 0.1 M acetate buffer showed a log *D* of 2.61 and –1.4 at pH 7.4 and 5.0, respectively.²⁵ An estimated log *P* value of 3.99^{26,27} and a Clog *P* value of 4.936 have been reported, using software modeling.²⁸

pK_a

Amodiaquine is a diprotic weak base with a pK_a of 7.08 and 8.14 at 25°C.^{25,29}

Dosage Form Strengths

The WHO treatment guidelines for malarial therapy recommend administration of artesunate along with ADQ for effective therapy of uncomplicated

Table 1. Concentration of Dissolved Amodiaquine Hydrochloride Achieved in Various Media at 37°C and the Corresponding (Maximum) Dose/Solubility (D/S) Ratios Based on the Maximum Tablet Strength (153 mg)^a and on the Maximum Recommended Single Dose (600 mg)^b

Medium	pH	Concentration (mg/mL)	D/S Ratio $D = 153$ mg Base ^c	D/S ratio $D = 600$ mg Base ^c
Water	6.4	8.1282	<24.60 ^f	<96.49 ^f
SGF _{sp} ^d	1.0	6.8653	<29.13 ^f	<114.25 ^f
SGF _{sp}	1.2	8.6740	<23.06 ^f	<90.70 ^f
Acetate buffer	4.5	8.8669	<22.55 ^f	<88.45 ^f
SIF _{sp}	6.8	3.1773 ^g	62.94	246.85
SIF _{sp} ^e	7.0	0.5303 ^g	1478.96	—
SIF _{sp} ^e	7.5	0.0848 ^g	9243.65	—

^aMaximum tablet strength listed in the WHO Essential Medicine List.

^bHighest single dose recommended in the SmPC.

^c153 mg of amodiaquine base is equivalent to 200 mg of amodiaquine hydrochloride.

^dSGF_{sp} with pH adjusted to 1.0.

^eSIF_{sp} with higher buffer concentration (80 mM) and pH adjusted to 7.0 and 7.5.

^fAs all ADQ dissolved in this medium, the D/S represents a maximum value.

^gThe concentration value indicates the equilibrium solubility of the API.

P. falciparum malaria.¹⁰ Because ADQ is generally administered along with artesunate, it is available in coblistered packs as kits and additionally as fixed dose combinations (FDCs). The FDCs are formulated as bilayered tablets with ADQ in one layer and artesunate in the other layer. Coblistered ADQ tablets having marketing authorization in Vietnam are available in dose strengths of 153 and 300 mg base.³⁰ The WHO prequalified product list includes three coblistered tablet formulations of ADQ and artesunate.³¹ According to the information in the prequalification dossier, ADQ coblistered and FDC products are licensed/registered in Benin, Republic de Guinea, Gabon, Senegal, Burkina Faso, Cameroon, Ghana, Kenya, Uganda, South Africa, Democratic Republic of Congo, and other African countries.^{32,33}

PHARMACOKINETIC PROPERTIES

Absorption and BA

The absolute BA of amodiaquine is not known; the API is not recommended for intravenous (i.v.) use as slow i.v. injection causes a decrease in the systolic pressure.³⁴ Amodiaquine is rapidly absorbed from the gastrointestinal tract and undergoes extensive first pass metabolism in the liver to ADQm. On administration of a single oral dose of 600 mg, Winstanley et al.³⁵ reported a maximum plasma concentration $3(C_{\max})$ of 32 ± 3 ng/mL after 0.5 ± 0.3 h and an area under the curve ($AUC_{0-\infty}$) of 154 ± 38 ng h/mL for amodiaquine. In the same study, ADQm had a C_{\max} of 181 ± 26 ng/mL, which was five times higher than that of the parent drug, at 3.4 ± 0.8 h. The $AUC_{0-\infty}$ of the metabolite in the plasma was reported as 8037 ± 1383 ng h/mL.³⁵ Single oral doses of 200, 400, and 600 mg showed a linear relationship between the dose and the AUC of ADQ and ADQm in six healthy subjects, indicating linearity of PK over this dose range.³⁶ The metabo-

lite, which retains microbial activity, has a plasma half-life ($t_{1/2}$) of 9–18 days in contrast to the parent drug, which has a $t_{1/2}$ of only approximately 3 h.^{37,38} Fortin et al.³⁹ reported a low intrasubject variability ($n = 26$) in C_{\max} and AUC of between 18% and 22% for amodiaquine and 8% and 16% for ADQm.³⁹

Winstanley et al.⁴⁰ found no significant difference in the C_{\max} and $AUC_{(0,6)}$ of amodiaquine between healthy subjects ($n = 7$) and Zambian and Nigerian malaria patients ($n = 10$ and $n = 4$). However, the time to attain maximum plasma concentration (T_{\max}) in patients was higher (1.75 ± 1.2 h) in comparison with that in healthy subjects (0.5 ± 0.1 h).⁴⁰

Administration of ADQ/artesunate FDC tablets along with a high-fat meal in healthy human volunteers slightly delayed and increased the C_{\max} and AUC of ADQ and its main metabolite.⁴¹ The C_{\max} and $AUC_{(0-t)}$ for ADQ were increased by 23% and 58%, respectively, as compared with the fasted state, whereas that for ADQm increased only by 18% for C_{\max} and 12% for $AUC_{(0-t)}$. Conversely, the C_{\max} and $AUC_{(0-t)}$ for artesunate decreased by 66% and 13%, respectively, as compared with the fasted state.⁴² The delay in T_{\max} for ADQ can be attributed to slower gastric emptying, whereas the higher C_{\max} and AUC values might be due to better solubilization of the weak base in the fed state. Indeed, precipitation at pH simulating the fasted intestinal state had been observed in the *in vitro* solubility studies. Because BE could not be demonstrated when the drug was administered in fed and fasted state, the product leaflets recommend against the administration of combination ADQ and artesunate formulations after a high-fat meal.^{32,33}

Permeability

Amodiaquine hydrochloride is reported to be readily absorbed from the gastrointestinal tract.⁵ However, literature searches on the absolute BA or fraction absorbed retrieved no results.

Hayeshi et al.²⁶ evaluated the permeability of amodiaquine through Caco-2 cells, having transepithelial electrical resistance (TEER) 6 of 300 Ω cm², and validated by [¹⁴C]-mannitol (1 μ Ci/mL) as a monolayer marker. The API demonstrated an apparent permeability coefficient (P_{app}) of 9.8×10^{-6} cm/s (a – b) and 3.86×10^{-6} cm/s (b – a) through the cells (personal correspondence with author). Although the authors claimed the drug would be completely absorbed, the interpretation was based on the API demonstrating a P_{app} value of $>1 \times 10^{-6}$ cm/s²⁶ and not according to the US FDA approved method of comparison with both highly and poorly permeable reference compounds evaluated in the same study.

Winstanley et al.⁴³ performed mass balance and tissue distribution studies of ¹⁴C-labeled amodiaquine in male Wistar rats following oral (8.6 mg/kg) and i.v. (3.8 mg/kg) administration. The oral dose size was chosen to reflect that used in clinical practice (equivalent to 600 mg amodiaquine base to a 70 kg man). Following oral administration of [¹⁴C] amodiaquine, 86 \pm 8.3% of the dose had been excreted by 72 h of which 77 \pm 9% was in the feces, 7 \pm 1% in urine, and 2 \pm 2% in cage washes. Although the authors reported almost comparable results for fecal and urinary excretion after the i.v. and oral administration of ADQ (102.6 \pm 9.7% of the i.v. dose was excreted by 72 h of which 90.9 \pm 9.6% was in the feces, 10.9 \pm 0.8% in urine, and 0.5 \pm 0.2% in cage washes), contribution of unabsorbed radiolabeled amodiaquine after oral administration to the fecal radioactivity cannot be ruled out.⁴³

Distribution, Metabolism, and Elimination

Amodiaquine is distributed extensively in the body. White et al.³⁴ reported an apparent volume of distribution (V_{ss}) of 17.4 L/kg (range 2.3–59.8 L/kg) in six healthy subjects following i.v. administration. In malarial patients, an even higher V_{ss} of 38.3 L/kg (range 4–128 L/kg) was observed at steady state.³⁴ Using ¹⁴C-labeled amodiaquine, Winstanley et al.⁴³ demonstrated accumulation of the drug specifically in the liver, kidneys, spleen, and bone marrow in rats, which are also sites of observed toxicity in man. ADQm showed a high blood to plasma ratio of approximately 3:1⁴⁰ as compared with the amodiaquine in healthy subjects. The high concentration of amodiaquine and ADQm in the blood is because of the accumulation of these two entities in the white blood cells and not in the erythrocytes.^{44,45} This was also demonstrated *in vitro* by studying its disposition in human neutrophils.⁴⁶ Interestingly, on comparison of the PK of ADQm in healthy and malarial patients, the patients showed a low ratio of 0.8:1 at the start of the study, which eventually rose to 3.04:1 as the parasitemia was cleared.⁴⁰

In vivo ADQ is chiefly metabolized in the liver to ADQm, which possesses antimalarial activity at about half the level of the parent drug.⁴⁷ Hydroxydesethyl amodiaquine⁴⁸ and bis-desethylamodiaquine³⁷ have also been identified as metabolites. Amodiaquine is rapidly cleared from the plasma and is detectable in the plasma for not more than 12 h because of the hepatic metabolism. The elimination half-life of ADQm is far longer (97.5 \pm 77.7 h), which probably reflects a low clearance and/or extensive distribution.⁴⁵ Amodiaquine and ADQm, both demonstrate protein binding to the extent of 90%–95%.⁴⁷

Less than 2% of the administered dose of ADQ is detected in the urine,^{35,45} indicating hepatic metabolism to be its major route of elimination.^{34,47} The main metabolic pathway in the liver is through the cytochrome P450, CYP2C8 isoenzyme.^{11,49} Several alleles of CYP2C8 have been identified, namely CYP2C8 1*–4*, of which CYP2C8 2* shows an almost six-fold lower metabolizing capacity than CYP2C8 1*. *In vitro* experiments with microsomal enzymes show that CYP1A1 and CYP1B1 are also found to contribute to the metabolism of amodiaquine.^{50,51} These enzymes, which are present in the extrahepatic tissues, could play a role in extrahepatic metabolism. However, their significance in the *in vivo* disposition of amodiaquine has not yet been demonstrated.^{51,52}

Additionally, a lower systemic clearance value [5.5 L/(kg h); range, 1.6–17.3 L/(kg h)] was observed in patients ($n = 10$) with *P. falciparum* infection in comparison with that [13 L/(kg h); range 4.7–56.61 L/(kg h)] observed in healthy subjects ($n = 7$).³⁴ The probable cause for the reduced clearance can be attributed to the impairment of the hepatic elimination capacity or a reduced hepatic blood flow in severe malaria.⁴⁷

DOSAGE FORM PERFORMANCE

Bioequivalence

Literature research on BE of ADQ products retrieved several studies indicating the test product to be bioequivalent to the comparator. As amodiaquine/artesunate combinations are recommended for malarial therapy, the test products were either ADQ tablets, coblistered with artesunate tablets, or were formulated as a FDC product. Table 2 indicates that ADQ products demonstrated BA parameters comparable with that of Flavoquine[®] (Sanofi Aventis, Paris, France), the comparator product suggested in the prequalification program of the WHO. The 90% confidence interval (CI) C_{max} and AUC values of the test product were within the BE acceptance range of 80%–125% except for the Artesunate Amodiaquine Winthrop (Maphar, Sanofi-Aventis Maroc, Morocco) FDC product, where a wider limit of 75%–133% was applied. A wider limit was allowed for this product

Table 2. Bioequivalence Study Results of Amodiaquine Hydrochloride Tablets

References	Dose	Subjects	Formulation	Composition	Prandial	Study Design	Pharmacokinetic Parameters	Bioequivalence Criteria, Statistics	Results	In Vitro Tests
Fortin et al.	400 ADQ	Data of 26 subjects were analyzed, healthy male Indian volunteers	Amonate FDC (Daifa Pharma, Turnhout, Belgium), Flavoquine® (Sanofi Aventis)	Not reported	Overnight fasting, no food allowed 2 h after tablet administration	Randomized, open label, two period, two treatment, two sequence, cross over design	C_{max} , t_{max} , AUC_{0-t} , $AUC_{0-\infty}$, $t_{1/2}$	Two one-sided test approach proposed by Schuurmann (1987), 90% CIs, analysis of variance (ANOVA)	The FDC product was bioequivalent to Flavoquine® (Sanofi Aventis)	Amonate released 92% ADQ in 30 min. Conditions of the test were not specified.
WHO Public Assessment Reports (MA047)	200 mg ADQ	44 healthy subjects	ADQ tablet coblistered in Falcimon Kit (Cipla, Goa, India), Flavoquine® (Sanofi Aventis)	Falcimon ADQ tablet excipients: colloidal anhydrous silica, magnesium stearate, maize starch, and pregelatinized starch with hydroxypropylmethyl cellulose and polyethylene glycol 6000 in film coating Flavoquine® (Sanofi Aventis): Excipient not reported	Overnight fasting	Randomized, single dose, open label, crossover	AUC, C_{max} and T_{max}	90% CIs, ANOVA	Bioequivalent	Not reported
WHO Public Assessment Reports (MA001)	200 mg ADQ	43 healthy subjects	ADQ coblistered tablet in artesunate tablets and amodiaquine hydrochloride tablets USP (Ipeca Laboratories Ltd., Mumbai, India), Flavoquine® (Sanofi Aventis)	Coblistered AQ tablet excipients: colloidal silicon dioxide, lactose monohydrate, magnesium stearate, maize starch, methyl paraben, propyl paraben, purified talc, sodium starch glycolate, and Tween 80	Overnight fasting	Randomized, single dose, open label, crossover	AUC, C_{max} and T_{max}	90% CIs, ANOVA	Bioequivalent	Not reported

Continued.

Table 2. Continued

References	Dose	Subjects	Formulation	Composition	Prandial	Study Design	Pharmacokinetic Parameters	Bioequivalence Criteria, Statistics	Results	In Vitro Tests
WHO Public Assessment Reports (MA058)	270 mg ADQ [2 FDC tablets and 31/2 Flavocquine® (Sanofi Aventis) tablets of 153 mg AQ]	32 healthy subjects	Artesunate Amodiaquine Winthrop (uncoated bilayered IR tablets), Flavocquine® (Sanofi Aventis)	Flavocquine® (Sanofi Aventis): Excipient not reported Bilayered tablet contained calcium carbonate, croscarmellose sodium, magnesium stearate, maize starch, microcrystalline cellulose, povidone, and colloidal anhydrous silica	Overnight fasting	Parallel design	AUC, C _{max}	90% CIs, statistical method not stated.	Amodiaquine was reported to show comparable bioavailability. However, wider limits of 75%–133% were applied	Not reported

as it was a new combination product with a different ratio of artesunate to ADQ, and hence, the criterion applied was to establish comparable BA supportive of the clinical efficacy and safety data, rather than to establish BE *per se* (personal correspondence). No reports of ADQ bioequivalence were found in the open literature.

Excipients

Antacid containing products such as magnesium trisilicate and kaolin are known to decrease gastrointestinal absorption of amodiaquine when administered simultaneously.¹¹ Preformulation studies performed by Kauss et al.⁵³ on artesunate/amodiaquine fixed combinations indicated an absence of excipient interactions between amodiaquine/artesunate blends and povidone (PVP K30), croscarmellose (Acdisol®), colloidal silicon dioxide (Aerosil® 300), and magnesium stearate.⁵³

The effect of excipients affecting the permeability or gastric transit time of the API cannot be detected by the biowaiver surrogate methods. Hence, such excipients, if present in the comparator, should be identical qualitatively and, as far as possible, quantitatively in the multisource products. Table 3 indicates the excipients, along with their respective amounts, which are utilized in immediate release (IR) formulations containing ADQ as the only active ingredient. Of these excipients, only polysorbate 80 is suspected to affect permeability, and none of these excipients are known to affect motility when employed in usual amounts.

Dissolution

The USP recommends 900 mL water as the medium for dissolution testing of ADQ tablets using apparatus 2, with an agitation speed of 50 rpm. The *Q* limit specified is $\geq 75\%$ drug release in 30 min.⁵⁴ Several studies were found in the open literature where dissolution studies were performed according to the USP specifications. Minzi et al.⁵⁵ reported almost 100% release of the API in 30 min from the Camoquin® tablets. Similarly, USP conform dissolution studies on locally produced amodiaquine tablets in Ghana by Owusu-Ansah et al.⁵⁶ revealed that all the formulations released approximately 100% of the labeled content within 30 min.

The USP method fails to reflect the formulation behavior over the whole range of upper gastrointestinal pH and, as it uses an unbuffered medium, is not a BCS-conform dissolution method. Therefore, additional dissolution tests (as specified in the WHO biowaiver guidelines) were performed with the pure API. These revealed that $>85\%$ of the drug was dissolved in 15 min at pH 1.2, 4.5, and 6.8, indicating that the drug fulfills the “very rapidly dissolving” criterion according to the WHO and EMA guidelines (Fig. 2). Because of the lack of availability of ADQ

Table 3. Excipients* Present in Amodiaquine IR Solid Oral Drug Products** from the WHO List of Prequalified Medicinal Products***

Excipient	Drug Products on the WHO List of Prequalified Medicinal Products ^a
Cellulose, microcrystalline	WHO ^{b,c}
Dextrin	WHO ^{b,c}
Hydroxypropyl cellulose	WHO ^{b,c}
Lactose	WHO ^d
Magnesium stearate	WHO ^{b-e}
Methyl parahydroxybenzoate	WHO ^d
Polysorbate 80	WHO ^{b-d}
Propyl parahydroxybenzoate	WHO ^d
Silica	WHO ^{d,e}
Sodium starch glycolate	WHO ^{b-d}
Starch	WHO ^{b-e}
Starch, pregelatinized	WHO ^e
Talc	WHO ^d

*Ingredients present in the coating are not included.

**Excluded are: combination products.

***Source of information: http://apps.who.int/prequal/whopar/whoparproducts/whopar_index.htm (Malaria products) (accessed: April 25, 2012).

^aFootnote by WHO: "Trade names are not prequalified by WHO. This is under local DRA responsibility. Throughout this WHOPAR the proprietary name is given as an example only".

^bAmodiaquine hydrochloride tablets.

^cARSUAMOON (amodiaquine 150 mg + artesunate 50 mg tablets).

^dArtesunate tablets and amodiaquine hydrochloride tablets USP.

^eFalcimon Kit (amodiaquine 153 mg tablets + artesunate 50 mg tablets).

products from regulated sources, dissolution was not performed on drug products.

Lacaze et al.⁵⁷ performed dissolution of artesunate-ADQ bilayer tablets and Flavoquine[®] (a Sanofi Aventis product) in acetate buffer pH 5.5 at 50 rpm. The dissolution profile indicated that the formulations released more than 85% of ADQ in 15 min from these formulations.⁵⁷ By contrast, dissolution studies carried out by Odunfa et al.⁵⁸ to evaluate the pharmaceutical equivalence of five amodiaquine brands marketed in Nigeria (Camoquin[®], USA; Larimal[®], India; Timec[®], India; Loquine[®], England;

Dart[®], Nigeria), using a basket apparatus rotated at 50 rpm in 900 mL 0.1 M hydrochloric acid, reported a maximum release of only around 80% of the labeled amount in 30 min for all the products. The difference in the amount released could be because of the use of a lower agitation speed (50 rpm) in the study instead of 100 rpm, the commonly recommended agitation speed for the basket assembly. No BE testing of the products were reported in either study.

DISCUSSION

Solubility

The solubility data available in open literature provided no data on the pH-dependent solubility behavior of ADQ. Hence, additional BCS-conforming solubility studies were performed on the API according to the WHO guidelines. The API demonstrated D/S values <250 mL over the pH range of 1.2–6.8 (Table 1), thus indicating it to be a "highly soluble" API according to the WHO guidelines. ADQ also fulfilled the EMA specifications for "highly soluble" API but demonstrated a borderline D/S value at pH 6.8. However, the API failed to qualify for the "highly soluble" API criteria according to the US FDA, according to which the above stated D/S limits need to be met over the pH range of 1–7.5, for the highest dose strength.

Permeability and Absorption

No data were available in the open literature, which unequivocally demonstrated the permeability of amodiaquine to be $\geq 85\%$. Although Hayeshi et al.²⁶ inferred complete absorption of amodiaquine based on the results obtained by Artursson and Karlsson⁵⁹, according to which drugs that are completely absorbed have a P_{app} value greater than 1×10^{-6} , the study did not validate the Caco-2 cell monolayer method according to the US FDA guidelines.

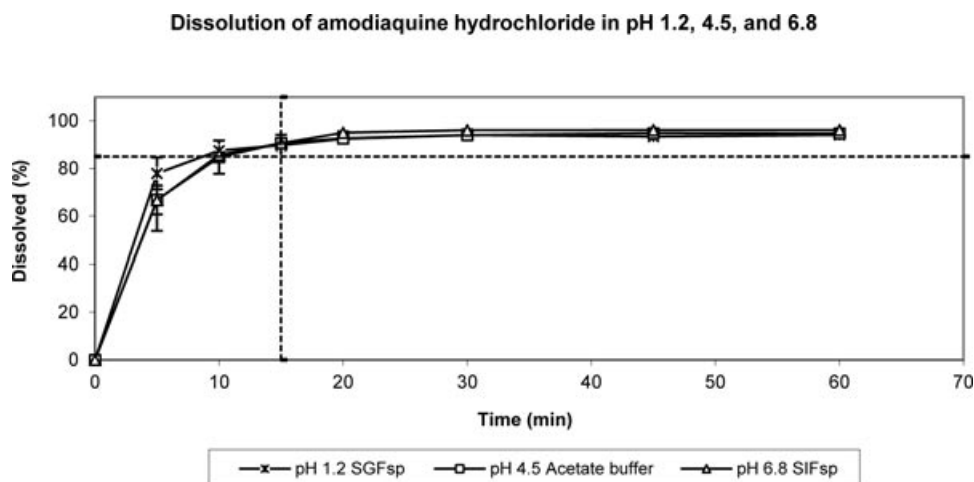


Figure 2. Dissolution profiles of 200 mg amodiaquine hydrochloride at pH 1.2, 4.5, and 6.8.

Yee⁶⁰ studied the Caco-2 permeability of several compounds and compared these with their *in vivo* absorption. The study concluded that the extent of drug absorption can be appropriately predicted to a great extent by the P_{app} values of the APIs, which are not P-glycoprotein substrates. On the basis of these results, ADQ would probably be classified as a drug with moderate permeability because the study indicated compounds that were 20%–70% absorbed *in vivo* demonstrated a P_{app} values between 1 and 10×10^{-6} cm/s.⁶⁰

In summary, because of the lack of conclusive data, the permeability of ADQ cannot be unequivocally classified.

Biopharmaceutics Classification System

The BCS categorizes APIs on the basis of its permeability and solubility into four distinct classes. Although the biowaiver procedure put forth by the WHO, US FDA, and EMA are all based on this system, the solubility and permeability criteria differ somewhat across the three guidances. On the one hand, WHO and EMA classify APIs as “highly soluble” if the D/S \leq 250 mL across a pH range of 1–6.8, whereas, US FDA requires this criterion to hold over a pH range of 1–7.5. There are also differences in the definition of dose. The dose considered by the WHO is the highest strength of the IR solid oral dosage form stated in the WHO Essential Medicines List for the API, that is, 153 mg in case of amodiaquine base. The US FDA guidance similarly defines the dose as the highest dose strength available. By contrast, the EMA defines the maximum dose of the API recommended in a single administration, as stated in the SmPC (600 mg of amodiaquine base).

As is evident in Table 1, ADQ easily fulfilled the “highly soluble” criteria according to WHO guidelines and was also able to meet the EMA specifications. However, at higher pH, the D/S ratio was above the specified 250 mL, thus failing to meet the US FDA criteria of high solubility across pH 1–7.5. However, it is noteworthy that pH 7.5 represents the distal part of the small intestine, which is unlikely to play a significant role in ADQ absorption. Hence, the solubility at this higher pH to the BCS is perhaps less relevant.

The Biopharmaceutics Drug Disposition Classification System (BDDCS) classifies drugs into four groups similar to BCS but replaces the permeability criteria with the major route of elimination. Thus, BDDCS Class I compounds are designated as “high solubility and extensive metabolism,” Class II as “poor solubility and extensive metabolism,” Class III as “high solubility and poor metabolism,” and Class IV as “poor solubility and poor metabolism.”⁶¹ Along these lines, if the principles of the BDDCS are applied on ADQ, its extensive metabolism *in vivo* (2% excretion of unchanged drug through urine) and a positive food effect

would hint at “high permeability” of the drug substance.

On the basis of the solubility data and inconclusive permeability data, ADQ is conservatively classified as a Class III API according to the EMA and WHO guidelines and Class IV according to the current US FDA specifications.

Risk of Bioequivalence Caused by Excipients and/or Manufacturing Parameters

The choice of excipients in a formulation plays a significant role in the BA of the drug and thus can affect BE of the products either by affecting drug dissolution or altering the gastrointestinal permeability. No reports suggesting interaction of ADQ with excipients were found in the open literature.

As the API itself fulfilled “very rapidly dissolving” criteria at the recommended dissolution pH, any excipient or manufacturing variables adversely affecting dissolution from drug products should be easily detected by the surrogate tests. However, dissolution tests are obviously not capable of indicating the effect of excipients, which alter gastrointestinal permeability and/or motility. In the absence of excipients that affect drug permeability or gastrointestinal motility, the absorption kinetics of Class III APIs would depend on the formulations and physiological factors.⁶² For almost all excipients listed in Table 3, the risk of a bioequivalent product passing the surrogate test specifications appear to be rather low if used in standard amounts. If polysorbate 80 is present in the comparator, it should be also used in the test product in an equal amount.

In the case of FDC products containing ADQ, the biowaiver decision also depends on the other API(s) present in the formulation. Preformulation studies performed on artesunate ADQ combinations revealed incompatibility between the two APIs in the presence of high humidity (moisture level $>1\%$) and at elevated temperatures. Failure of artesunate to sustain chemical integrity during the classical wet granulation process in the presence of ADQ led to the development of bilayer FDC formulations where the two APIs were separated into different layers of the tablets. In view of this stability issue, it is suggested that the stability aspect of an ADQ/artesunate FDC be more thoroughly evaluated before a biowaiver is considered for FDC products.

Patients Risk's Associated with Bioequivalence

In 1989, the WHO Expert Committee recommended ADQ to be discontinued from prophylactic and therapeutic use against malaria, due to the fatal agranulocytosis and hepatotoxicity shown by travelers during prophylactic therapy.⁶³ Later, in 2000, the 19th WHO Expert Committee on Malaria Treatment recommended the use of this compound, but only for

malarial therapy.⁶⁴ Presently, the 2010 Guidelines for Treatment of Malaria propose use of amodiaquine/artesunate combinations for the treatment of uncomplicated malaria caused by *P. falciparum*.¹⁰ The risk associated with bioequivalent levels of the drug, given the “chequered” history of amodiaquine, needs to be carefully considered.

The risk associated with a bioequivalent product can arise from either subtherapeutic or suprathereapeutic levels of drug in the patients. As is the case with every anti-infective agent, subtherapeutic levels would not only subjugate the effectiveness of therapy but also encourage the development of resistant strains of organisms.

By contrast, a suprathereapeutic concentration could precipitate adverse effects. In consideration of the latter, it must be noted that amodiaquine therapy, in recommended doses, does not show clinically significant concentration-related adverse effects. The tolerability of amodiaquine/artesunate combination therapy was assessed from the results of 31 comparative and noncomparative studies including 5333 patients over 19 countries (mainly African). These demonstrated an absence of treatment or drug-related fatal cases.⁶⁵ A few incidences of mild neutropenia and increased serum alanine aminotransferase levels (an indicator of hepatotoxicity), although rare and statistically insignificant, were reported during therapy. All these conditions were asymptomatic and normal levels were achieved once the therapy was completed.^{52,66–68} Because the nature of the hematologic and hepatotoxic side effects of amodiaquine appears to be idiosyncratic in nature,^{69,70} the risk of these effects appearing as a result of administration of a bioequivalent product seems very low.

Another scenario where suprathereapeutic ADQ levels can be encountered is on coadministration with high-fat meal. It was observed that high-fat meal increased the C_{max} and $AUC_{(0-t)}$ values of ADQ and ADQm in comparison with the fasted state. The 90% CIs of the ratio of fed versus fasted C_{max} and $AUC_{(0-t)}$ were outside the BE range.⁴¹ Hence, a biowaiver for ADQ products (as single API) can be considered only if both test and comparator leaflets state that the product should not be taken with high-fat meals.

CONCLUSIONS

Solubility studies on ADQ revealed that the API conforms to the “highly soluble” criteria according to WHO and EMA but not with respect to the US FDA specifications. The permeability of ADQ is not conclusively demonstrated in the literature. As a result, the API can be conservatively classified as a Class III API according to WHO and EMA specifications but Class IV with respect to the US FDA criteria.

Considering the principles of a biowaiver approval, the IR solid oral dosage forms of ADQ can be approved with a “biowaiver procedure” according to WHO and EMA guidelines, provided the multisource products contain excipients (and in similar amounts) present in products approved by the International Conference of Harmonisation or associated countries (also see below for polysorbate 80) and that the labeling specifies that the product should not be coadministered with high-fat meals.

Additionally, if excipients such as polysorbate 80, which are known to influence motility/permeability, are used in the comparator, they should be present in the same quantity in the test as in the comparator product. Both, the test and the comparator products, need to comply with the BCS-conforming dissolution test criteria for “very rapidly dissolving products” ($\geq 85\%$ drug release in 15 min at pH 1.2, 4.5, and 6.8) in line with the conservative BCS Class III. If any of the above-mentioned criteria cannot be met, BE should be demonstrated in a PK study.

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