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Fixed artesunate–amodiaquine combined pre-formulation study for the treatment of malaria

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ABSTRACT

Artemisinin-based combination therapies, including artesunate (AS) + amodiaquine (AQ), are the currently recommended first-line treatment of uncomplicated falciparum malaria. Fixed-dose co-formulations offer logistic and adherence advantages. This paper reports the initial research phase of the pre-development process of an AS–AQ formulation, further developed by the Drug for Neglected Diseases Initiative (DND*i*). Results demonstrate that AS and AQ are not compatible, and AS degradation is related to three main parameters: water content (>1%), elevated temperature (80 °C in dry condition) and possibly the 4-aminoquinoline moiety. Furthermore, AS and AQ incompatibility led to AS degradation and pharmaco-technical changes in classical wet granulation tablets. Both active principles are stable as dry powders. These investigations led to further development of various co-formulations, including the bilayer tablet currently on the market.

1. Introduction

Artemisinin and its derivatives artesunate (AS), artemether, artemotil (aka arteether), and dihydroartemisinin (DHA) are potent antimalarial drugs, but cannot be used alone in practice because of their short plasma half-life, requiring unsuitably long treatment courses. They are therefore used in combination with other, longer-lived antimalarial drugs (artemisinin-based combination therapies, ACT). The ACT strategy was tested and developed in the 1990s at the Thai-Burmese borders, later extended to other areas (Adjuik et al., 2004), and is currently recommended by the World Health Organization (WHO) for the treatment of uncomplicated malaria in all malaria endemic countries (Ruttimann, 2006; WHO, 2006). The artemisinin component of the ACT produces a rapid reduction of the parasite biomass while the companion drug clears the remaining parasites; combining drugs with different targets offers mutual protection against resistance. One of these ACTs is AS combined with amodiaquine (AQ). AS and AQ was shown to be generally effective and safe for the treatment of uncomplicated malaria in Africa (Zwang et al., 2009).

AS and AQ was used initially as a combination of the two individually formulated products, then as co-blistered products and more recently as a fixed-dose co-formulation (Ndiaye et al., 2009; Sirima et al., 2009).

The co-blistered products, dosed by age, have been associated with suboptimal dosing (Beer et al., 2009; Brasseur et al., 2009). A fixed-dose bilayer combination was developed by DND*i* (Drugs for Neglected Diseases *initiative)* under the Fixed-dose Artesunate Combined Therapy (FACT) project which included as partners WHO/TDR (which funded among other things the initial formulation studies), the University Sains Malaysia and the University of Bordeaux 2 (Croft, 2005). The product is currently WHO-prequalified and is marketed by Sanofi-Aventis as Artesunate–Amodiaquine Winthrop® or ASAQ® and Coarsucam®. Drug disposition after intake of the fixed product is similar although not entirely bioequivalent to the separate products (Navaratnam et al., 2009). The dosage of the ASAQ fixed-dose product is based on weight-for-age data (Taylor et al., 2006).

The main pharmaceutical issue with AS-containing formulations is stability. In aqueous solution AS is rapidly converted to DHA, with a half-life of 26 min and 10 h at pH 1.2 and 7.4 respectively at room temperature (Haynes, 2006). Both AS and DHA decompose readily under aqueous acidic conditions to provide significant amounts of the peroxyhemiacetal, which, like DHA, decomposes to the inert end product 2-deoxyartemisinin under acidic or basic conditions (Haynes et al., 2007). Furthermore, AS is not compatible with basic quinolines (Haynes, 2006).

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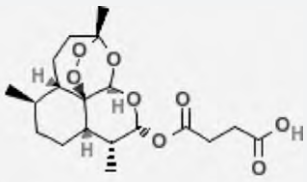
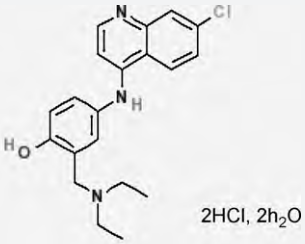
Table 1
Main artesunate and amodiaquine characteristics.

	Artesunate (AS)	Amodiaquine (AQ)
		
Chemical structure	$C_{19}H_{28}O_8$	$C_{20}H_{22}ClN_3O.2HCl.2H_2O$
Molecular weight	384.4	428.79
Macroscopic aspect	White microcrystalline powder	Bitter yellow microcrystalline powder
Maximum of absorption (UV)	202 nm	222, 236, 241 nm[a]
X-rays diffraction	Crystalline structure	Crystalline structure
Melting point (DSC)	145 °C	157.5 °C
Water content		
Karl Fischer	0.4% (w/w)	8.54% (w/w)
Desiccation	0.3% (w/w)	0.8% (w/w)
Mean particle size	140.5 μm	12.8 μm
Specific surface	0.127 m^2/g	1.55 m^2/g
Content in raw material	99.8%[b]	99.57%[a]

[a] Certificat d'analyses no. 604 (Amodiaquine Chlorhydrate batch no. 1003QJJ), Parke Davis Senegal.
[b] Certificate of Analysis Artesunate, batch no.1.03, Knoll, Liestal, Switzerland.

This paper reports on the extensive investigations made during the early phases of the project on ASAQ pre-formulation feasibility, the first step towards the development of a new antimalarial drug combination. Initially, both active principles were characterized for their physicochemical properties, their compatibility and identification of the key parameters of ASAQ degradation. The main pharmaco-technical features of tablet formulation are also reported.

2. Materials and methods

2.1. Chemicals

AS was purchased from Knoll, Switzerland (bought since by Abbott Laboratories) and amodiaquine hydrochloride (AQH) salt (AQ·2HCl·$2H_2O$) and amodiaquine base (AQb) were obtained from Parke Davis, Senegal. DHA was a generous gift from Knoll (Switzerland). Quinine was purchased from Sigma–Aldrich, France. Solvents and buffers were of analytical grade. Tablet excipients were purchased from Cooper, France and Seppic, France. Before starting pre-formulation studies, the physicochemical properties of AS and AQ were characterized (Table 1).

2.2. Analytical methods

Several analytical methods were developed for the purpose of this study. Active principle blend and tablet content were determined using HPLC with UV detection for AS and UV spectrometry for AQ. Direct UV absorption could not be used for AS analysis, since AS degradation products had similar UV spectra and hence could not be distinguished. Degradation products were investigated using thin layer chromatography (TLC). Details of each method are summarised in Table 2.

2.3. AS solubility determination

Solubility of AS was determined in various media using European Pharmacopoeia apparatus 2 (Sotax AT7, Switzerland) at 100 rpm. The test was carried out at defined temperature (22 °C or 37 °C) with an excess of AS (3 g) in 1000 ml of dissolution medium for 1 h (extended dissolution test led to AS degradation and DHA formation). At the end, dissolution medium was paper filtered. Samples were analysed using HPLC method previously described for AS determination (Table 2).

Following media were tested: distilled water (at 22 °C), hydro-alcoholic solutions (ethanol 95°/distilled water 10/90, 20/80, 30/70, 60/40% (v/v) at 22 °C), sodium acetate buffer (0.1 M $CH_3COONa_3H_2O$, CH_3COOH for pH adjustment to 5, 5.5, 6.0, tested at 37 °C), pharmacopoeia phosphate buffer pH 7 (at 37 °C) and 0.1 M HCl (at 37 °C).

2.4. Powder blend compatibility study

2.4.1. Initial screening of AS powder stability in various conditions

Powder blends of AS ±4-aminoquinoline (AQ base or hydrochloride, or quinine) in various proportions (1/1 or 2/0.71) ± 5% (w/w) water or 5% (w/w) ethanol 95° were prepared and divided in two flasks and crimped, one kept at 25 °C and the other at 55 °C. The morphological and colour changes were initially checked daily using the following qualitative scale: 0 = no modification, + = mild modification, ++ = moderate modification, +++ = significant modification. Practically, morphological appearance was powder (0), sticky powder (+), thick paste (++), liquid (+++) and sample colour yellow (0), light brown (+), brown (++), dark brown to black (+++). After 14 days, blends were analysed by TLC.

In order to investigate the effect of water on AS stability, AS and AQ dry blend (1/1) was divided into 11 flasks, and

Table 2
Analytical methods used for artesunate and amodiaquine analysis.

Analytical method/active principle	Artesunate	Amodiaquine
TLC		
Silica gel (0.25 × 20 × 20 cm)	Detection after 1 h at 150 °C at 366 nm Rf AS = 0.40–0.43 LOD AS = 200 ng Rf DHA = 0.89–0.92 LOD DHA = 100 ng	Detection after 5 min at 120 °C, at 366 nm Rf AQ = 0.75 LOD AQ = 100 ng
Acetonitrile–water–NH_3 150:30:7.5% (v/v)	Rf AM LOD AM = 350 ng	
UV spectrophotometry	Not appropriate	λ = 342 nm
HPLC		
Column: Hypersil ODS C18, 5 μm, 125 × 4.6 mm Mobile phase: Na acetate buffer 50 mM pH = 5/CH_3CN (60/40%, v/v); flow 1.5 ml min^{-1} Oven: 35 °C 50 μl injection UV detection: 211 nm Internal standard: AM	Tr AS = 5.1 ± 0.1 min LOD AS = 200 ng Tr AM = 7.90 ± 0.1 min	Not determined

Notes: TLC: thin layer chromatography; HPLC: high performance liquid chromatography; Rf: resolution factor; Tr = retention time; AS: artesunate; DHA: dihydroartemisinin; AM: artemisinin; AQ: amodiaquine.

increasing quantities (0.5%) of water were added from 0 to 5% (w/w).

2.4.2. DSC (differential scanning calorimetry) analysis

DSC thermograms of AS, AQ, tablet excipients, as well as their binary physical mixtures were performed using Mettler Toledo equipment (TC15 differential scanning calorimeter, DSC30 oven and STARe 9.01 software). Samples of 6–8 mg were sealed in aluminium foil and analysed from 30 to 250 °C at 5 °C/min.

2.4.3. TLC analysis of the effect of water on stability of drugs and excipients

Five percent of water (w/w) was added to dry mixtures of each drug (AS or AQ) with each excipient (PVP K30®, Acdisol®, magnesium stearate, Aerosil® 300) in 15/80 (% w/w) proportions. The total mass of 10 g was split into two vials, crimped and stored at 25 or 55 °C for 14 days. Then, any visual modification was noted and 100 mg of AS-excipients or AQ-excipients mixtures were extracted using 2 ml of toluene or 2 ml of ethanol/water (50/50) respectively. After 5 min of ultrasounds and 5 min of centrifugation at 4000 rpm, 10 μl of each supernatant was analysed by TLC comparing to AS, AQ, artemisinin and DHA standards at 10 mg/ml.

2.5. Tablet preparation and evaluation

2.5.1. Tablet preparation

Tablets were prepared by wet granulation technique (Table 3). Sodium croscarmellose (Acdisol®) was used as diluent/desintegrant; polyvinylpyrrolidone (PVP K30®, 10% aqueous solution) was used as a binder, and a mixture of colloidal silica (Aerosil® 300) and magnesium stearate (1:2) was used as lubricant. AS, AQ and a part of Acdisol® (2.26%, w/w) were mixed in a planetary mixer and moistened with PVP solution. The moist mass was then passed through 1.6 mm mesh of oscillating granulator and dried in fluid bed drying (Retsch, TG 100, Germany) at 60 °C for 30 min. After drying, granulation was screened again (0.71 mm mesh), mixed with lubricants and 1% (w/w) of Acdisol® before compression on a single punch tablet press (Korsch Pressen, type EK 0, Germany) using 13 mm flat die-punch.

2.5.2. Tablet dissolution test

In vitro drug release tests were conducted using European Pharmacopoeia apparatus 2 (Sotax AT7, Switzerland) at 50 rpm, series connected to circulation vials of UV spectrophotometer (mc2, Safas, Monaco) used for AQ dosage. The test was carried out at 37 °C in 900 ml sodium acetate buffer pH 5.5 (0.1 M $CH_3COONa_3H_2O$, 0.1 M CH_3COOH, NaOH for pH adjustment). The choice of pH 5.5 dissolution medium was based on two reasons: (i) to mimic physiological conditions at the artesunate absorption site in the upper part of intestine (Batty et al., 1998) and (ii) this condition was found to be the best compromise for stability/solubility for both tested drugs, AS (an acid) and AQ (a base).

At defined times (*T*0, 10, 20, 30, 45, 60 min) samples (1 ml) were withdrawn using pre-filter (10 μm) equipped syringe. Samples were added the internal standard solution (artemisinin 0.2 mg/ml in acetonitrile; 3/1) and analysed using the HPLC method previously described for AS determination (Table 2).

2.5.3. Tablet sample preparation for drug content analysis

AS tablet content: Five tablets were crushed in a mortar and their mean weight of powder introduced in 50 ml flask, completed with acetonitrile to extract AS. After 15 min of stirring, the obtained suspension was filtered (nylon 0.45 μm filter) and diluted 1/5 in HPLC mobile phase and artemisinin (internal standard) added. 50 μl of prepared sample was injected three times for AS analysis.

AQ tablet content: Two tablets were sonicated with ultrasounds in 500 ml 0.1% HCl. The obtained suspension was filtered and then diluted 1/100 with HCl 1%. AQ was dosed using UV spectrophotometer method (see Table 2 for description).

2.6. Statistical analysis

Student's unilateral test was used to check the probability for the percentages to be based on comparable mean values. Results were considered significantly different with a probability of error of $p < 0.05$.

3. Results and discussion

3.1. AS solubility

AS room temperature water solubility was found to be 0.296 mg/ml. When tested over a range of pH conditions and ethanol content in aqueous dissolution medium, AS solubility increased with pH, as AS is a weak acid, yet limited (<0.31 mg/ml) (Fig. 1A) and the addition of significant ethanol

Table 3
Tablet formulation.

Formulation	Weight per tablet (mg)	% (w/w)
Amodiaquine 2HCl, $2H_2O$ (corr. to AQ base)	352.64 (270)	70.96 (54.33)
Artesunate	100.00	20.12
Na croscarmellose	16.22	3.26
Polyvinylpyrrolidone	20.76	4.18
Mg stearate	4.90	0.99
Colloidal silica	2.45	0.49

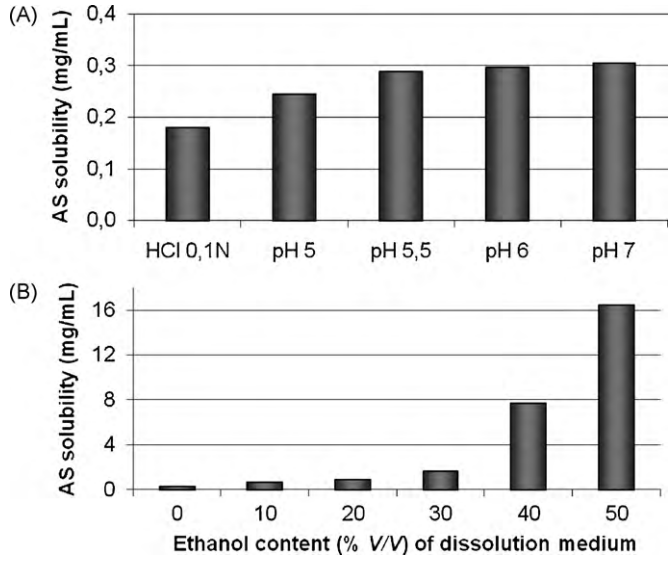


Fig. 1. AS solubility in various dissolution media: (A) as a function of pH at 37 °C and (B) as a function of ethanol content at 22 °C.

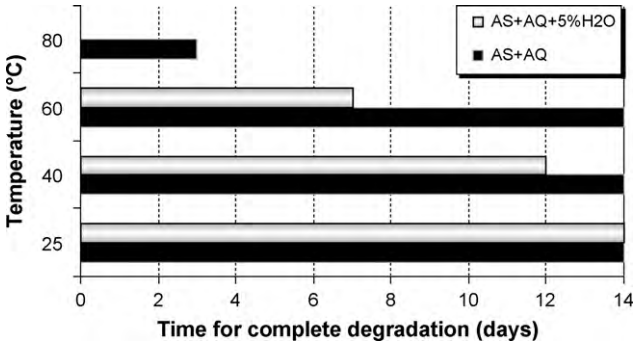


Fig. 2. Effect of temperature and humidity on AS instability in ASAQ blend.

quantities to the medium was needed to enhance dissolution (Fig. 1B).

3.2. Identification of AS degradation parameters

Preliminary TLC results showed that AS is degraded to DHA in the presence of AQ under stress conditions. To understand the mechanism of this decomposition and identify the key factors involved, several AS/AQ power blend compatibility studies were conducted. Powder blends were observed visually (morphological or colour change) and analysed by TLC at 14 days. The objective of these studies was not to characterize the kinetics of AS degradation (defined elsewhere; see Haynes, 2006), but to identify critical conditions for AS degradation to avoid during formulation.

There were three main parameters affecting AS stability (Fig. 2): presence of basic quinolines (like AQH), temperature and water

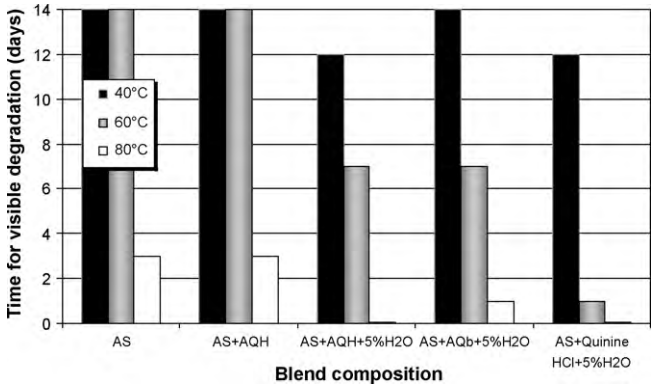


Fig. 3. Influence of AQ hydrochloride (AQH), AQ base (AQb) and quinine on AS blend stability.

(humidity). Each of these parameters was studied to identify AS degradation risks.

Water presence (5%, w/w) for 14 days did not induce AS degradation at room temperature, but as temperature increased, AS stability in the presence of water decreased. When water was replaced by ethanol, no AS accelerated degradation was observed even in the presence of AQH.

Increasing quantities of water were added to AS + AQH blend and analysed at room temperature or 55 °C. No degradation was observed at room temperature for any water proportion after 14 days. Visible changes at 55 °C (consistency and colour) correlated well with TLC degradation: the more AS was degraded, the more the blend darkened and powder liquefied. After 14 days, at least 1% water was needed for partial AS degradation in the presence of AQH at 55 °C. In blends containing 4–5% water, AS was fully degraded at the end of the study at 55 °C (Table 4).

Temperature was another important factor for AS degradation. However, moderate temperature alone was not enough as the AS/AQH blend remained stable at up to 60 °C throughout 14 days (Fig. 2). A temperature of 80 °C was necessary to obtain AS degradation in powder blend without adding water. Nevertheless, in the presence of 5% of water, temperature affected AS stability from 40 °C.

Therefore, both humidity and temperature concur to AS degradation. This observation was confirmed by HPLC analysis of AS + AQH and AS + AQH + 5% water blends after 14 days at 55 °C, where 100% of AQH was found in both conditions, 100% of AS in condition without added water whereas no AS was detected in the presence of water.

The presence of AQH accelerated AS degradation (Fig. 3). Despite no visible difference between AS alone and AS + AQH at 40 °C and 60 °C (colour/consistency change), complete AS degradation was revealed by TLC after 14 days at 80 °C in the presence of AQH, but not with AS alone. These effects were also seen in the presence of 5% water at 60 °C.

To identify more precisely the cause of AS/AQH interaction, two other blends were prepared: AS + AQ base (AQb), to study the effect of hydrochloride salt *vs.* base, and AS + quinine, to

Table 4
Stability of AS in AS + AQ HCl blend as a function of water quantity added; visible changes (colour and consistency) of powder blend and TLC results (0 = no degradation, + = slight; ++ = medium; +++ = complete degradation).

	Water added (%, w/w)	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
7 days	Visible degradation	0	0	+	+	+	+	+	++	++	++	++
14 days	Visible degradation	0	0	++	++	++	++	++	++	+++	+++	+++
		Yellow blend								Dark brown blend		
	TLC detected degradation	0	0	+	+	+	++	++	++	+++	+++	+++
		No degrad. product, AS fluorescence normal		Degradation product Rf = 0.92 increasing AS fluorescence diminishing						Degrad. product Rf = 0.92, No AS spot detected		

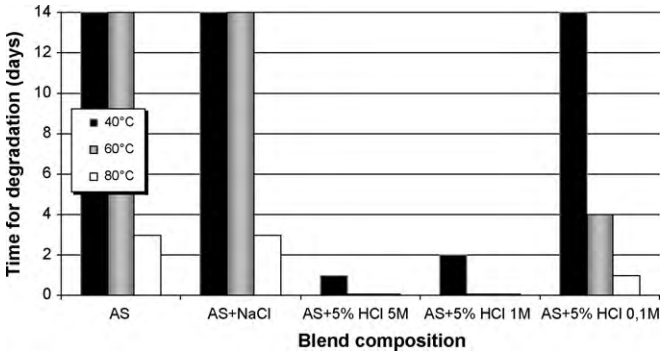


Fig. 4. AS stability in the presence of Cl^- and free HCl.

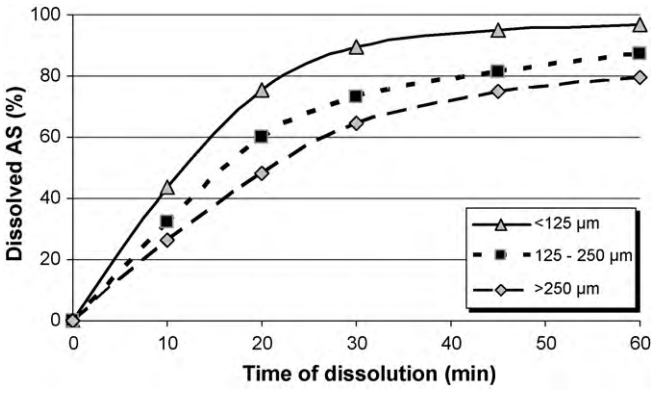


Fig. 5. Dissolution profiles of AS tablets as a function of AS particle size.

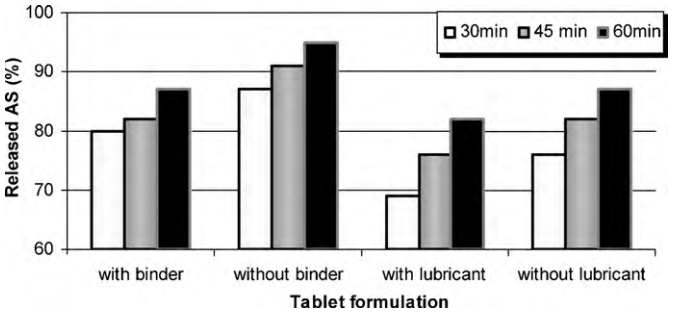


Fig. 6. Influence of tablet formulation on AS tablet release at 30, 45 and 60 min of the dissolution test.

investigate the role of the 4-aminoquinoline moiety in AS degradation.

The speed of degradation was AQb < AQH < quinine (cf. Fig. 3). However, an effect of HCl itself on AS degradation could not be completely excluded. Therefore, the influence of free HCl and NaCl was studied. Chlorides did not affect AS stability and TLC analysis showed the isolated AS spot up to a temperature of 60 °C (Fig. 4). However, AS degradation was accelerated as soon as free hydrochloric acid was added in a concentration-dependent manner. Therefore, AS degradation could be accelerated by HCl release from AQH under stress conditions (temperature, humidity), consistent with the pH dependency of AS degradation (Haynes, 2006; Olliaro and Navaratnam, unpublished).

In conclusion on AS/AQ interaction, AS itself was found to be unstable under conditions of humidity (≥1% added water) but stable to temperature changes in dry conditions (80 °C needed for degradation without added water). The presence of AQH accelerates degradation, probably due to the presence of the 4-quinoline nucleus with the contribution of the release of free HCl from AQH under conditions of high temperature and humidity. Furthermore, AS was previously shown to be unstable in pharmaceutical solvents (Gaudin et al., 2007). Based on these observations, a solid oral form was considered the best option in order to avoid AS instability conditions. Tablet formulation prepared in controlled humidity atmosphere (<1% humidity) and kept in aluminium blisters appeared to be the most cost-effective manufacturing process.

3.3. AS/AQ tablet formulation and key parameters for drug release

To further investigate tablet parameters, a basic tablet formulation containing AS and AQH was prepared by wet granulation (Table 3). The aim of this study was to verify (i) whether AS could tolerate a classical tablet preparation without degrading and (ii) to identify the critical conditions for the formulation process.

Before starting tableting, the absence of interaction caused by excipients was checked using differential scanning calorimetry. No interaction was observed in powder mixtures of drugs (AS or AQH) and excipients (PVP K30®, Acdisol®, Aerosil® 300, Mg stearate).

Like in pre-formulation study conditions, no interaction occurred at room temperature while moistening the AS/AQH blend: the tablet wet granulation procedure did not induce immediate AS/AQH degradation.

The influence of some critical parameters affecting drug release was then studied, i.e. AS particle size, tablet formulation (namely presence of binder and lubricant), and tablet hardness.

AS particle size appeared to be an important factor for tablet dissolution (Fig. 5; $p \leq 0.015$). This characteristic should therefore be part of the specifications and validation process when acquiring AS raw material. Homogenous particle size distribution <250 µm of diameter was desirable for reproducible drug dissolution profiles.

Furthermore, as expected according to the physical roles for binder (PVP K30®) and physicochemical properties for lubricant (colloidal silica/Mg stearate), i.e. their binding property and hydrophobicity respectively, the presence of these constituents in tablet formulation significantly delayed AS tablet release (Fig. 6; $p < 0.003$ and $p < 0.005$ for binder and lubricant respectively). Hardness did not influence significantly the kinetics of release (60 N was not different from 115 N).

Table 5
45-min dissolution test results of ASAQ tablets after 3 months normal and acceleration aging conditions.

Released PA after 45 min (% T0 content)	Amodiaquine		Artesunate	
Time of storage (months)	T0	3 months	T0	3 months
25 °C/60%RH open vial	93.5 ± 2.0	95.1 ± 0.7	93.9 ± 2.6	95.2 ± 1.1
25 °C/60%RH closed vial	93.5 ± 2.0	96.2 ± 0.1	93.9 ± 2.6	95.6 ± 1.1
40 °C/75% HR closed vial	93.5 ± 2.0	96.8 ± 1.8	93.9 ± 2.6	86.5 ± 1.1[a]
40 °C/75% HR open vial	93.5 ± 2.0	93.4 ± 2.6	93.9 ± 2.6	77.3 ± 1.7[b]

[a] 86.5% of T0 AS content corresponded to 95% of total AS content after 3 months degradation.
[b] 77.3% of T0 AS content corresponded to 83% of total AS content after 3 months degradation.

Then, the stability of the tablets was evaluated for 3 months in regular (25 °C/60%RH (residual humidity)) and accelerated (40 °C/75%RH) conditions. Drug content, dissolution rate, tablet hardness and mean tablet weight were verified monthly (Figs. 7 and 8 and Table 5). The effect of keeping the tablets in Bakelite stoppered glass bottles *vs.* open glass vials was studied.

AQ content was stable for 3 months whereas AS content decreased at 40 °C/75%RH after 2 months in open vial and at 3 months in closed vial for 6.6 and 9.2% respectively (cf. Fig. 7). Storage in glass stoppered bottles did not protect AS from degradation ($p = 0.28$).

Three months of accelerated conditions also modified AS dissolution kinetics (Table 5; $p < 0.0002$). The decrease in AS open vial release could not be explained by AS degradation alone, as only 83% of the initial AS content (corresponding to 77.3%* of theoretical AS content, see Table 5) was found after 45 min. On the contrary, in closed vials, AS degradation explains AS release as 86.5% of T0 AS content corresponded to 95% of total AS content after 3 months degradation. Open vial storage significantly decreased AS dissolution release compared to closed vial storage in accelerated aging condition ($p < 0.001$). AQ drug release was not modified.

Physical parameters (Fig. 8) were also modified in accelerated stability conditions. Tablet hardness in open vials first increased but then decreased significantly ($p = 0.005$), whereas the mean tablet weight increased, indicating humidity uptake as the cause of tablet consistency and mass changes. In closed vials tablet hardness increased moderately ($p = 0.045$), whereas the mean tablet weight increase was not significant. In regular stability conditions, tablet hardness modification did not exceed 61 ± 9 N and was not statistically different between open and closed vials. Slight tablet mean weight modifications observed were not significant.

In view of these results concerning tablet conditioning, Bakelite stoppered glass bottles delayed but did not prevent the occurrence of pharmaco-technical changes and AS degradation. Desiccant con-

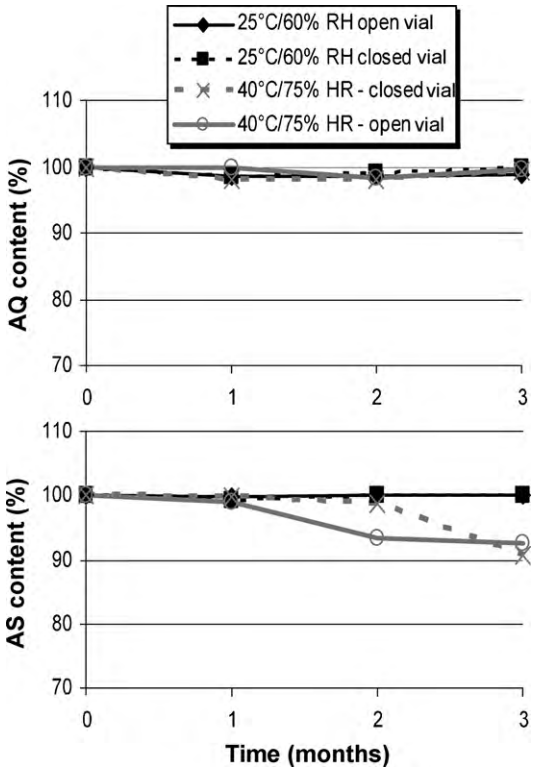


Fig. 7. Drug content stability during 3 months in normal (25 °C/60%RH) and accelerated aging conditions (40 °C/75%RH).

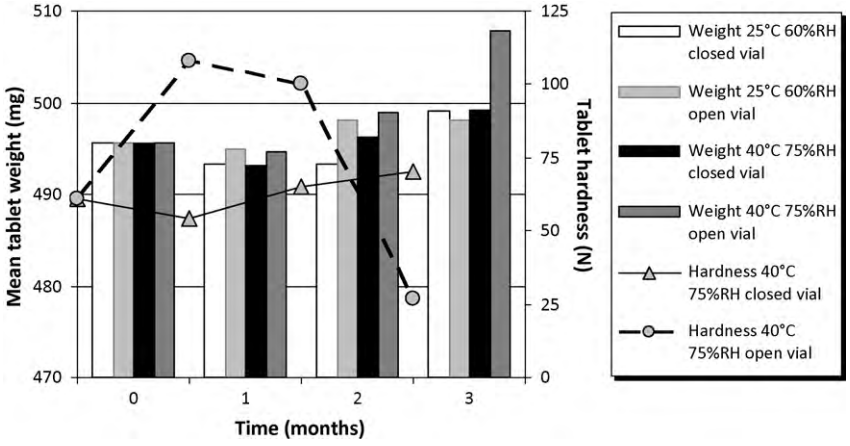


Fig. 8. Mean tablet weight (left *Y* axis, bars) and tablet hardness (right *Y* axis, lines) during 3 months of normal condition storage (25 °C, 60%RH) or accelerated aging condition storage (40 °C, 75%RH) in either open or closed glass vials.

taining or moisture-resistant containers like aluminum foil should be considered for packaging.

4. Conclusion

These results demonstrate that AS and AQ are not compatible in the presence of humidity and high temperature. This incompatibility appears to be related to the quinoline structure, but other quinoline and non-quinoline moieties should be further studied to confirm this hypothesis. ASAQ tablets manufactured under classical wet granulation process did not stand the preliminary accelerated stability studies (3 months at 40 °C, 75%RH). By contrast, no AS degradation occurred in unformulated AS/AQ powder blends as long as water presence was lower than 1% (w/w).

Therefore, unformulated APIs can be stored either separately or together provided humidity is controlled. The stability of fixed-dose co-formulations should be tested when manufactured under humidity-controlled conditions and packaged in moisture-resistant containers. However, the presence of AQ increases the risk of AS degradation as soon as water >1% is present. Therefore, it would be advisable to opt for dry formulations whereby the active ingredients are physically separated, prepared in controlled humidity atmosphere (<1% humidity) and kept in aluminium blisters.

Eventually, after formulation development, for this co-formulation a bilayer tablet was developed to limit the physical contact between the APIs, but is technically more demanding and more expensive. The formulation of solid oral forms manufactured under strictly inert or humidity-controlled atmosphere could be an economically viable alternative for a low-cost product that can be afforded in resource-poor settings while satisfying stability requirements.

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