

Preliminary formulation of a fixed-dose paediatric combination of artesunate and amodiaquine hydrochloride

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Abstract

Background. Since the introduction of artemisinin combination therapy (ACT), it has been recognised that challenges exist in presenting drugs as fixed dose combinations due to potential incompatibilities of the different chemical compounds. The aim of this study was to develop stable prototype formulations combining Artesunate (ART) and Amodiaquine hydrochloride (AMQ).

Materials and Methods. Two fast-disintegrating granular formulations, containing ART and AMQ respectively were produced by wet granulation. Samples were stored as single component formulations or blends in glass vials for periods up to 13 weeks at refrigerated storage conditions (10°C), room temperature (24-26°C) and ambient humidity (in the dark and light conditions), 25°C/75% RH, and 50°C/75% RH. The active agent content of the two drugs was determined using HPLC-UV at 1, 4 and 13 weeks from the start of the study. Statistical analysis of data were undertaken to determine the factors influencing the stability of the formulations, both alone and in combination.

Results. The chemical stability of ART was markedly affected by relative humidity, with greatest levels of degradation occurring at 13 weeks after storage at 50°C/75% RH. No significant loss of active agent content was observed for AMQ at any storage conditions over the duration of the study.

Conclusions. The results indicate that stable fixed dose granular formulations of ART and AMQ can be produced that are stable under accelerated conditions. These formulations should, however, be protected from extremes of relative humidity using suitable packaging materials to avoid degradation of ART.

1 Introduction

Combination therapies are becoming important options for the treatment of malaria owing to their capacity to address issues of parasitic resistance to the older and affordable antimalarial drugs such as chloroquine and sulphadoxine-pyrimethamine. This resistance has led to increasing rates of morbidity and mortality due to malaria especially among children less than five years of age [1]. Artemisinin Combination Therapy (ACT) is basically the combination of artemisinin or any of its derivatives with another class of antimalarial drug(s), either as fixed dose combination or multi-drug therapy [2]. The artemisinin derivatives are very active anti-malarial drugs and can produce large reductions (up to 10,000 fold) in parasite biomass per asexual cycle, thereby reducing malaria transmissibility [3]. The unique features of ACT are associated with the features of the artemisinin component and include: rapid and substantial reduction of parasite biomass; rapid parasite clearance; rapid resolution of clinical symptoms; effective action against multi-drug resistant *Plasmodium falciparum*; reduction of gametocyte carriage, which potentially reduces transmission of resistant

alleles [2]. The immediate effect of the artemisinin component is to reduce the parasite biomass making the residual biomass to be exposed to maximum concentration of the partner drug, well above its minimum inhibitory concentration, resulting in a lesser likelihood for resistant mutations to occur [2].

Since the introduction of ACT, it has been recognised that challenges exist in presenting drugs as fixed dose combinations due to potential incompatibilities of the different chemical compounds, particularly the artemisinins. Compounds from this chemical series are thermally labile and chemically reactive and, therefore, might be prone to degradation in the presence of other drugs or during manufacturing [4]. It has, therefore, been advised to consider the use of a dual combination, in which formulations of each drug are made in such a manner that prevents direct interaction or are individually packaged for separate administration [4]. Fixed-dose combinations of ART and AMQ have been produced as bi-layer tablets through methods that are technically more demanding and expensive [5, 6]. Moreover, the facilities for producing bi-layer tablets are not readily available in third world countries, which are malaria endemic

areas, recognised as poor regions of the world. The study of Kauss *et al.*, revealed that the degradation of ART is associated with high temperature, humidity and probably 4-quinoline nucleus with the contribution of the release of free HCl from AMQ under conditions of high temperature and humidity [6]. Our previous study, however, showed an interaction between ART and water produced by the crystallization of AMQ, and not with the quinoline moiety [7].

The strategy adopted in this study was to formulate individual granules of ART and AMQ and mixing these granules according their required dose ratio. The method allowed minimal contact of both drugs, thereby seeking to slow down the rate of degradation of ART. We have developed a stable, prototype formulation combining ART and AMQ and identified factors leading to the instability of ART.

2 Materials and Methods

2.1 Chemicals and Reagents

Artesunate and amodiaquine hydrochloride were obtained from Mangalam Drugs (India) through ERICA Pharma (India). Mannitol was supplied by Roquette (UK), polyvinylpyrrolidone K29-32 (PVP) was obtained from BASF (Germany), croscarmellose sodium (Ac-Di-Sol[®]) was obtained from FMC Polymer (Republic of Ireland). Absolute ethanol analytical grade, ethanol HPLC grade, and acetonitrile HPLC grade were purchased from Fisher (UK). Aerosil 200 was purchased from Degussa (India), whilst potassium dihydrogen orthophosphate and orthophosphoric acid were obtained from BDH (UK).

2.2 Formulations

Fast disintegrating granules containing 50% w/w ART were prepared by the wet granulation method using polyvinylpyrrolidone (PVP) as binder (2% w/w), croscarmellose sodium (5% w/w) as disintegrant and mannitol (43% w/w) as diluent. The formulation ingredients were mixed in a dry mortar with the aid of a pestle. Different granulating liquids, water and ethanol, were used to evaluate their effects on ART stability. The wet mass was passed through a 1.7 mm sieve and then dried at 60°C for 20 min when ethanol was used and 60 min when water was used as granulating liquid. The dry granules were passed through 1.4 mm sieve.

AMQ granules containing 50% w/w drugs were also prepared using water as granulating liquid following the same method described for ART. A 1:4 mixture of ART:AMQ granules were made and stored in a sealed glass vials.

2.3 Chemical analysis

Granules containing ART and AMQ alone and granular blends (1:4) of ART:AMQ were analysed to determine the active agent content using modified HPLC assay method proposed in the International Pharmacopoeia for ART [8]. The HPLC system consisted of Hewlett Packard HPLC 1050 series, with an auto sampler, quaternary pump and programmable wavelength detector. The mobile phase was made up of acetonitrile: 50 mM phosphate buffer (50:50) pH 3.0 flowing through a Spherisorb[®] C18 (ODS2) column 250 × 4.6 mm i.d., 5 μm; injection volume is 20 μl.

2.4 Stability of preliminary formulations

The effects of light, temperature and humidity on the stability of ART and AMQ in the individual granules and in their granular mixtures were investigated. Samples were stored as the single component formulations and as granular blends of the two agents in glass vials for periods up to 13 weeks at refrigerated storage conditions (10°C), room temperature (20-25°C) and ambient humidity (in the dark and light conditions), 25°C/75% RH, and 50°C/75% RH. The active agent contents of the two drugs were determined using HPLC-UV at 0, 1, 4 and 13 weeks. During the stability studies, some data-points exceeded the 100% level by a range between 0.2 - 6%, which may be due to some error introduced in the course of the analysis.

3 Results & Discussion

3.1 Drug content

The uniformity of content of the granular formulations was established through evaluation of the active agent contents of the replicate samples (n=4). The average drug contents were 99.55±0.51% and 102.99±0.71% for ART and AMQ respectively. For the combined drug product, the average drug content was 102.2±3.30%.

3.2 Stability studies

Student t test was applied to determine the primary factors leading to degradation of the active agents. The results showed that humidity had a marked influence on ART stability, with degradation accelerated at high temperature. The effects of light and temperature on ART stability were only significant at the 13th week (Fig. 1). The most adverse condition leading to the rapid degradation of ART was 50°C/ 75% RH. Under these conditions, the combined effects of temperature and relative humidity appeared to be synergistic.

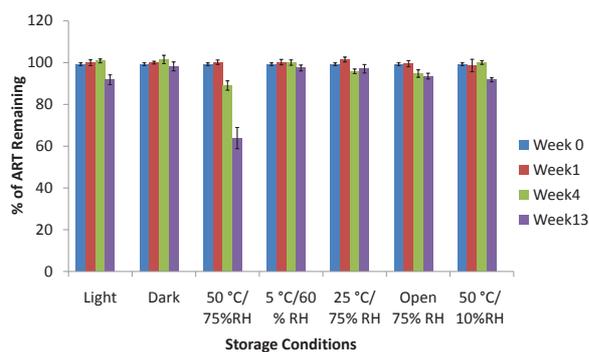


Figure 1. The stability of Artesunate in single formulation. Data show the mean percentage artesunate concentrations and standard deviations.

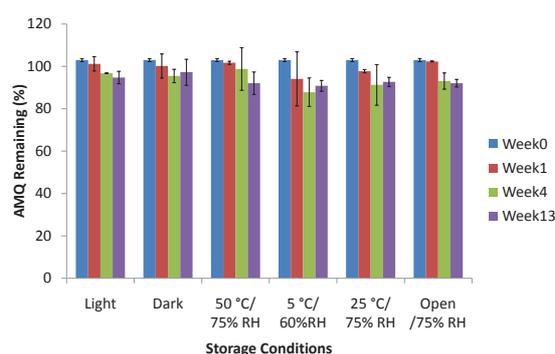


Figure 3. The stability of Artesunate-Amodiaquine granule mix. Data show the mean percentage drug concentrations and standard deviations.

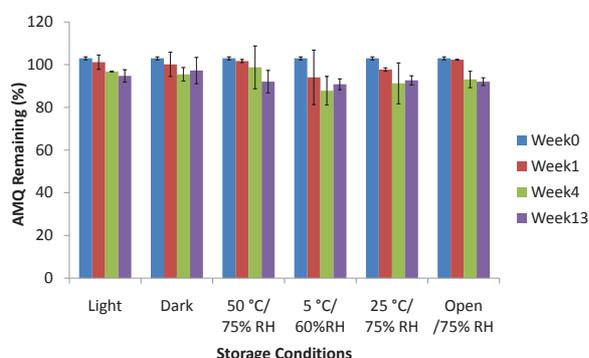


Figure 2. The stability of Amodiaquine in single formulation. Data show the mean percentage amodiaquine concentrations and standard deviations.

The physical stability of ART granules was poor when ethanol was used as granulating fluid, with caking of samples observed after 4 weeks. Caking of granules is an inherent problem associated with the use of mannitol as diluent. On the other hand, AMQ stability was adequate regardless of the storage conditions (Fig. 2), showing no significant loss of active agent content over the duration of the study.

The degradation of ART in the presence of high temperature and high relative humidity is an intrinsic property of ART; the formulation excipients as well as the method of formulation did not enhance the degradation [7]. The stability of the combined drugs in formulation indicated that the decrease in the drug content observed during storage at various conditions is as a result of the degradation of ART (Fig. 3).

4 Conclusions

Fixed-dose formulations of ART and AMQ can be produced by blending granular formulations containing the individual agents at the appropriate ratio. This could provide a flexible option for adjusting the dose for different age groups within the paediatric population. Humidity is recognised as the primary factor leading to the chemical instability of ART. These formulations should, therefore, be protected from extremes of relative humidity using suitable packaging materials

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