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Short communication

Formation and identification of a degradant in chlorproguanil–dapsone–artesunate (Dacart™) tablets

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ABSTRACT

Chlorproguanil hydrochloride, dapsone and artesunate are three compounds with anti-malarial properties developed as a triple combination drug product (Dacart™) for the treatment of malarial infections. During long-term stability studies, a degradant was observed which increased with time and had the potential to limit the shelf-life of the product. Through a combination of HPLC and spectroscopic analyses, the structure of the degradant was identified to be an adduct of a fragment of artesunate with dapsone. The response factor was determined to allow an accurate assessment of its levels in drug product. The likely mechanism for its formation is postulated to be *via* the water-mediated degradation of artesunate to give succinic acid followed by reaction of the liberated succinic acid with dapsone. The formation of this degradant demonstrates a potential stability risk for future combination therapies incorporating artesunate. These risks are particularly pertinent to products of this type given the climatic conditions which prevail in countries where such therapies are likely to be employed.

1. Introduction

Malaria is one of the largest causes of morbidity in the developing world with most deaths occurring among African children [1]. There are a wide number of treatments available for malarial infections [2] and artemisinin derivative therapies are currently the "gold standard" among these. However, drug resistance to commonly used anti-malarial drugs is increasing rapidly [3] and the World Health Organization (WHO) recommends that combination therapies are developed in order to minimize the potential for resistance to develop against artemisinin [4,5]. As part of this program, a triple combination therapy (Dacart™) was developed combining chlorproguanil hydrochloride, dapsone and artesunate (Fig. 1). The tablets were formulated as a fixed dose combination tablet for the treatment of acute uncomplicated *Plasmodium falciparum* malaria [6] on a not-for-profit basis in association with the Medicines for Malaria Venture (MMV) [7].

The proposed commercial formulation was a film-coated immediate release tablet with two dose strengths for oral administration to children and adults, respectively. The different amounts of each active pharmaceutical ingredient (API) in these formulations not only determine the product strength, but also influence the physical properties of the tablets and their behavior on stability. In formal stability studies, batches stored for 36 months in conditions 30 °C/65% relative humidity (RH) showed out-of-specification results for an unknown impurity peak with relative retention time (RRT) ~1.2 at a level of 0.3% area in pediatric formulations and 0.8% area in adult formulations, relative to dapsone. As the product was designed for the treatment of malaria in sub-Saharan Africa it was important that it had a long shelf life in ICH Zone 4A climates [8,9]. This was necessary to facilitate distribution and storage of the product in countries which have little infrastructure for controlled temperature distribution of drug products. The appearance of the new impurity above the specification limit suggested a potential problem with the proposed shelf life of the product and the requirement for further investigation of its structure and mechanism of formation. The structure of the impurity was therefore characterized using a combination of HPLC–mass spectrometry (LC–MS) and NMR spectroscopy.

2. Experimental

2.1. Formulation composition

Pediatric and adult strength tablets were both placed in the formal stability studies. Pediatric strength tablets contained 12 mg

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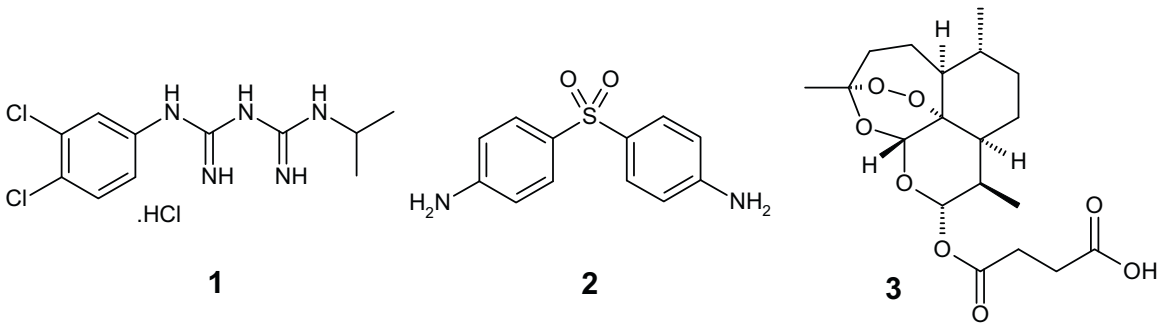


Fig. 1. Structures of chlorproguanil hydrochloride **1**, dapsone **2** and artesunate **3**.

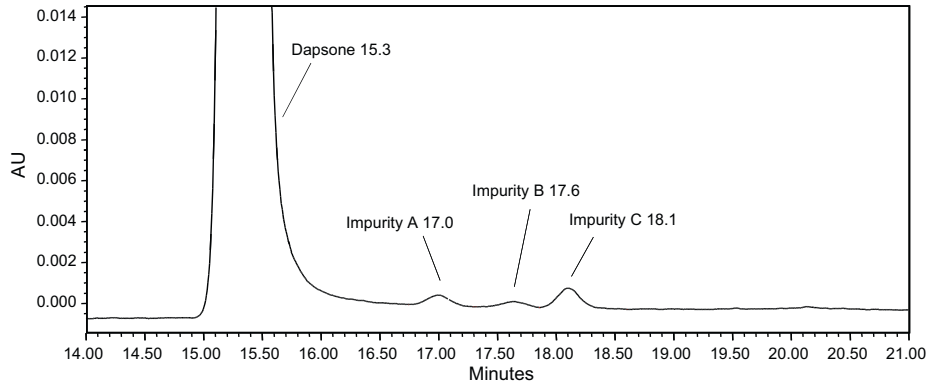


Fig. 2. Portion of the HPLC chromatogram of pediatric formulation tablet after 36 months stability showing dapsone, known impurity A and new impurities B and C formed by degradation.

chlorproguanil hydrochloride, 15 mg dapsone and 24 mg artesunate. Adult strength tablets contained 60 mg chlorproguanil hydrochloride, 75 mg dapsone and 120 mg artesunate. In addition to the active components, both formulations contained mannitol, microcrystalline cellulose, croscarmellose sodium, hypromellose, magnesium stearate and a distinguishing Opadry® colour.

2.2. Stability studies

Samples were stored at conditions of 30 °C/65% RH and sub-samples were taken for analysis at timepoints of 1, 3, 6, 12, 24 and 36 months to analyze for evidence of degradation. Sub-samples at each timepoint were analyzed by HPLC against a specification limiting not more than 0.2% of any new impurity.

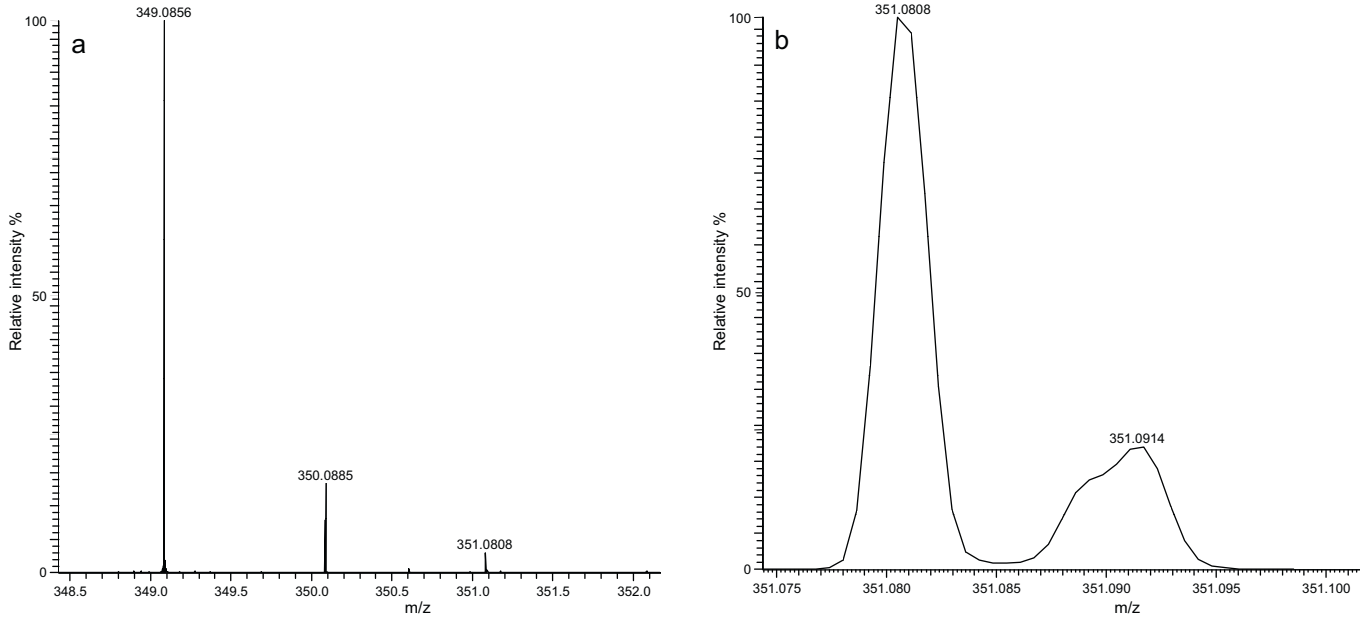


Fig. 3. (a) Mass spectrum of impurity C and (b) the expanded view of the M + 2 peak cluster showing the clearly resolved peak of ^{34}S at 351.0808 Da and the unresolved ^{18}O and $^{13}C_2$ isotope peaks.

2.3. Sample preparation

Samples were prepared for HPLC and LC–MS analysis by dissolution in a mixture of 200 mM ammonium acetate in water, methanol and water in a ratio of 1:1:8 (v/v/v).

2.4. High performance liquid chromatography (HPLC)

Analytical chromatographic separations were achieved using an Agilent 1100 HPLC system equipped with a Waters Spherisorb ODS1 5 μm, 4.6 mm × 150 mm column held at 25 °C. The binary mobile phase consisted of (A) ammonium acetate in water (pH 3.0, 20 mM) and (B) acetonitrile and was delivered at a flow rate of 0.7mL/min. Components were eluted in gradient mode starting at 16% B, reaching 18% B over the first 8 min, then 22% B over a further 8 min, 26% B over 7 min, 34% B over 7 min, 84% B over 5 min and holding for a further 5 min. Post-run equilibration time was 10 min. The injection volume was 40 μl for 60/75/120 mg (adult formulation) sample solutions and 50 μl for 12/15/24 mg (pediatric formulation) sample solutions. The detector wavelength was set at 260 nm.

2.5. HPLC–mass spectrometry (LC–MS)

LC–UV–MS data were obtained using an Agilent 1100 HPLC equipped with a diode array detector (DAD) connected to a Thermo LTQ Orbitrap mass spectrometer equipped with an electrospray ion source. HPLC conditions were reproduced as described in Section 2.4. Data were acquired in positive ion mode using a mass-to-charge ratio (m/z) range of 100–1000 at a resolution of 100,000 FWHM, source voltage of 3.5 kV and capillary voltage of 47 V. Fragmentation data were acquired using a normalized collision energy of 35% on m/z 349.

2.6. NMR spectroscopy

NMR spectra were recorded on a Bruker AV400 NMR spectrometer fitted with an HCFP QNP probe using standard pulse sequences. Spectra were recorded at 300 K with 32 k data points over a spectral width of 20 ppm and employed a thirty degree pulse width and recycle delay of 10 s to ensure complete relaxation between scans. 128 scans were acquired. Samples were prepared by dissolution in dimethyl sulfoxide-d_6 (DMSO-d_6) to a concentration of approximately 10 mg and used tetramethylsilane (TMS) as an internal reference (δ 0 ppm).

2.7. Impurity characterization

(4-({4-[(4-Aminophenyl)sulfonyl]phenyl}amino)-4-oxobutanoic acid) (**4**) is available commercially (CAS RN 5934-14-5). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.51 (m, 2H), 2.58 (m, 2H), 6.11 (s, 2H), 6.60 (d, 2H), 7.50 (d, 2H), 7.70–7.78 (m, 4H), 10.33 (s, 1H), 12.14 (br s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 28.4, 30.9, 112.5, 118.3, 125.5, 127.3, 128.7, 136.3, 142.4, 153.0, 170.5, 173.3. HRMS (ESI) m/z calcd. for $C_{16}H_{17}N_2O_5S$ $[M+H]^+$ 349.0853, obsd. 349.0860 (2.0 ppm error). MSMS (m/z, rel. int. %) (331.0748, 50), (249.0691, 100).

3. Results and discussion

In the formal stability studies, batches stored for 36 months in conditions 30 °C/65% RH were analyzed using the HPLC method described in Section 2.4 and showed two additional peaks, designated as impurities B and C, eluting after a known compound designated as impurity A (Fig. 2). Quantification of the two additional peaks revealed the later eluting of the two peaks, impurity C, to be the most abundant with absolute levels of 0.29% area and 0.78% area in the pediatric and adult formulations, respectively (Table 1). These results were above the specification limit of 0.2% area for any unknown impurity and hence it was required to identify the structure of the compound causing the out-of-specification result. No additional characterization of the minor component peak was required or attempted.

Table 1
Levels of impurities A, B and C in pediatric and adult formulations after 36 months stability determined by HPLC.

	Pediatric formulation	Adult formulation
Impurity A	0.14	0.08
Impurity B	0.09	0.13
Impurity C	0.29	0.78

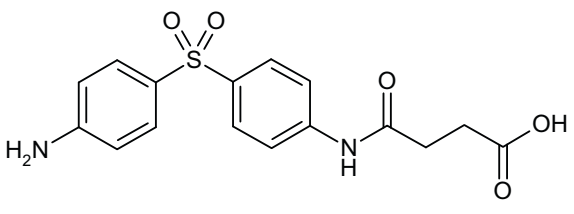
Mass spectrometry data of the peak due to impurity C showed a peak with m/z 349.0856 Da (Fig. 3a). Further study of the $[M+H]^+ + 2$ Da isotope peak showed the presence of a ^{34}S isotope peak at 351.0808 Da, confirming the presence of sulfur in this impurity. The measured mass of 349.0856 Da, together with the isotope pattern, was concordant with the expected value for a molecular formula of $C_{16}H_{17}N_2O_5S$ within an error of 1 ppm.

MSMS data showed the impurity to fragment by loss of a moiety consistent with $C_4H_5O_3$ to give a fragment ion with a mass equivalent to that of dapsone. The structure of the impurity was therefore postulated to be dapsone with an additional $C_4H_5O_3$, and it was proposed that this was in the form of an additional moiety of 4-oxobutanoic acid due to that moiety being present as part of the artesunate structure (Fig. 4).

A sample of this compound was obtained and its structure confirmed by NMR spectroscopy. The stability samples were spiked with this marker and showed co-elution of the marker and the impurity peak by LC–MS. Additionally, MSMS data of the impurity peak matched those of the marker, thus confirming the structure of the impurity as **4** (Fig. 4).

Structurally, **4** could form from a direct reaction of dapsone with artesunate or, more likely, through dapsone reacting with succinic acid which is a known degradant of artesunate when moisture is present and therefore likely to be present in the tablet at low level [10]. Additionally, the reaction of dapsone with succinic acid to give **4** releases a molecule of water, potentially promoting the further degradation of artesunate and hence the further formation of the degradant during longer periods of storage. To support this theory, the levels of the other product of such degradation, dihydroartemisinin (DHA), were seen also to increase over the course of the stability study.

In order to ensure accurate quantification of the level of the degradant in stability samples, its relative response factor (RRF) compared to dapsone was determined through a combination of HPLC and ^{1}H NMR spectroscopy. A sample of dapsone was spiked with **4** to give a mixture of the two species. The ^{1}H NMR spectrum of the mixture was obtained (Fig. 5) and through integration of the signals in the spectrum at 7.44 ppm (dapsone, 2H) and 7.51 ppm (**4**,



4

Fig. 4. Structure of degradant impurity 4-({4-[(4-aminophenyl)sulfonyl]phenyl}-amino)-4-oxobutanoic acid (**4**).

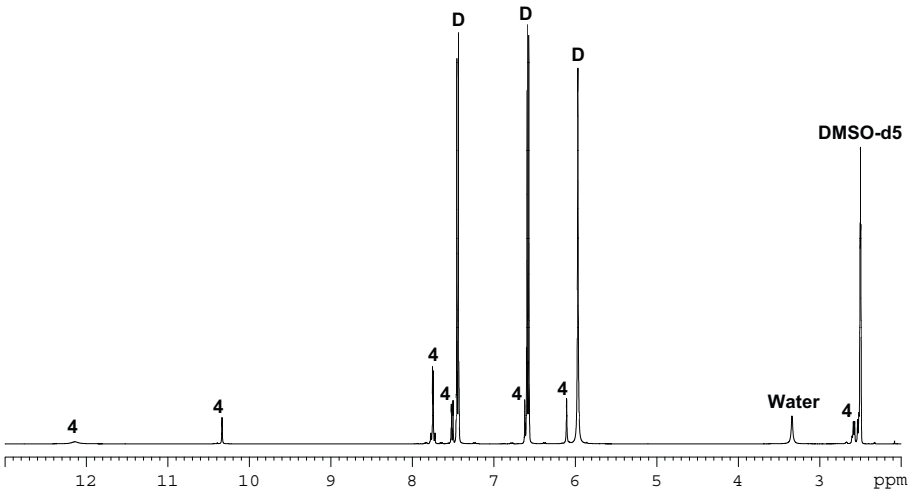


Fig. 5. ^{1}H NMR spectrum of dapsone (D) spiked with **4**.

2H), the level of **4** relative to dapsone was calculated as 22.0% w/w. The level of **4** relative to dapsone was also measured for the same mixture by HPLC giving a value of 25.7% area of **4** relative to dapsone. The RRF of **4** relative to dapsone was calculated using Eq. (1) to be 1.17. This value is considered within the range (0.8–1.2) approximating unity and hence the original values obtained for the levels of **4** in dapsone could be considered to be valid without the need for further adjustment. As a consequence, the stability data were taken forwards without modification and with the conclusion that there would still be a potential impact on the shelf-life that could be assigned to the product.

$$\text{Relative response factor (4 relative to dapsone)} = \frac{\%\ \text{w/w by NMR}}{\%\ \text{area by HPLC}} \quad (1)$$

4. Conclusions

Artemisinin-based products are currently the most effective treatments available to treat malarial infections and combination therapies of available anti-malarial drugs, such as Dacart™ (the development of which was terminated for unrelated reasons [11,12]), hold out the potential for more effective treatments in the future. The stability of products of this type is critical to their viability given the climatic conditions which prevail in countries where they are required. An understanding of the degradation pathways of the individual components and those arising from interactions between these components enables products to be developed with adequate shelf-lives for storage in conditions of relatively high temperature and humidity. Through a combination of HPLC, LC–MS and NMR analyses, a degradant impurity generated on stability testing of Dacart™ has been identified. The structure of the impurity leads to the potential conclusion that the shelf-life of this combination drug product may be limited to a period shorter than that desired. This observation of water-mediated degradation of artesunate to succinic acid and the subsequent reaction of the liberated succinic acid with, in this case, dapsone demonstrates a potential pathway for degradation of this product and hence potentially for other combination products incorporating artesunate. This possibility should be taken into account in the development of alternative combination products and treatments. The rate of degradation and the limiting implications that this has for the shelf-life of the product are relevant to others involved in the development of anti-malarial agents.

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