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Determination of chloroquine and its decomposition products in various brands of different dosage forms by liquid chromatography

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Abstract: Various commercial preparations of chloroquine dosage forms have been examined both by thin-layer chromatography (TLC) and liquid chromatography (LC). TLC showed that all these preparations yielded more than one spot, indicating possible degradation. An LC method has been adapted for the determination of chloroquine in these drug formulations. The standard calibration curve was linear over the concentration range 1–6 μg ml^{-1}. Chloroquine was assayed in various brands of different forms (ampoules, tablets and syrups). However, it was observed, for some samples, that the bands obtained were rather broad, showing shoulders or peak splitting, indicating the presence of other compounds coeluting with chloroquine. The utility of this method for the quality control of this major drug is assessed in the context of the need to carefully monitor drug purity in a tropical climate, particularly in situations where there may be doubt about the quality of the primary manufacturer.

Keywords: *Chloroquine; decomposition products; tropical stability; liquid chromatography; pharmaceutical dosage forms.*

Introduction

Chloroquine (Cq, Fig. 1) is by far the most extensively used chemotherapeutic and prophylactic drug for malaria. The drug has been used for over 30 years for the treatment of this disease, which is now endangering life and the economy in several developing countries. It is claimed that malaria in the Sudan is mostly due to infection with *Plasmodium falciparum* which is known to respond very well to Cq [1].

Until the 1960s and 1970s few brands of Cq from drug companies of international repute were available to patients suffering from malaria in the Sudan. However, in the last few years a large number of different dosage forms from different sources have been introduced. These include drug donations received in response to the starvation outcry.

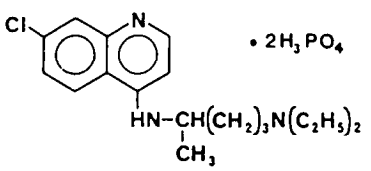

Figure 1
Chloroquine phosphate.

It has been noted that some patients do not apparently respond to treatment with Cq [2]. This led a group of workers to claim that the parasite may have developed Cq resistance [3]. It was also observed that patients suffering from malaria responded differently to drugs from different suppliers [2]. This was the background which led to the authors' interest in the problem of analysing Cq levels in dosage forms. Earlier work had focused on the separation and quantitation of chloroquine enantiomers by chiral LC [4].

The present investigation involved screening various brands from a number of different dosage forms by thin-layer chromatography to examine whether there were any detectable decomposition products. Adverse climatic conditions in the Sudan (high temperature and strong sunlight), poor storage, handling and transport were some of the important factors to be borne in mind in this context.

Therefore a high-performance liquid chromatographic method using hydroxychloroquine as internal standard was adapted [5] to measure the Cq content in a variety of Cq preparations available in the Sudan and to screen them for any degradation.

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Experimental

Materials

Chloroquine phosphate and hydroxychloroquine (internal standard) were kindly donated by Sterling Winthrop Pharmaceutical Company (Khartoum, Sudan). Tablets, syrups and ampoules of Cq of different brands were purchased from the local market. All chemicals and reagents used were of analytical grade and were used without any further purification.

Instrumentation

The liquid chromatography system consisted of a Reichelt Chemie Technik LC solvent supply, in conjunction with a Rheodyne Model 7010 injection valve equipped with a 50-μl loop. Detection was carried out using a RCT Thomachrom UV detector III (fixed wavelength at 254 nm). Chromatograms were recorded on a Reichelt Chemie Technik Omnicorder.

Chromatographic methods

Separations were carried out using a stainless-steel chromatographic column (250 × 4.6 mm i.d.) packed with 5-μm Spherisorb S5 ODS1 (Anachem). The mobile phase consisted of a mixture of 0.1 M phosphate buffer (pH 3.0)–acetonitrile (60:40, v/v). Potassium perchlorate (0.1 M) was used as an ion-pairing agent. The flow rate was 1 ml min^{-1} and the chart speed was 5 mm min^{-1}. All measurements were made at ambient temperature. Solutions were all freshly prepared and carefully protected from light during the experiments.

Thin-layer chromatography

TLC was carried out using silica gel type DG and the following solvent system as a mobile phase: ethylacetate–ethanol (absolute)–ammonia (s.g.0.880) (25:2:2, v/v/v). Experiments were carried out directly and on the chloroformic extracts of the basified solutions of standard Cq, syrups, tablets and chloroquine ampoules.

Liquid chromatography

A calibration curve for chloroquine phosphate was obtained by preparing six solutions each containing hydroxychloroquine as internal standard (3 μg ml^{-1}) and chloroquine phosphate over the range 1–6 μg ml^{-1}, respectively. Triplicate injections of each solution were made and the mean peak height ratios were measured and plotted against concentration. A straight line was obtained indicating that the method was linear over the concentration range concerned and had an insignificant intercept on the ordinate. The linear regression equation gave:

$$y = 0.24x + 0.0215 \ (n = 6; r = 0.995).$$

The bracketting method involved the injection of a standard solution containing chloroquine phosphate (5 μg ml^{-1}) in sequence with tests. This was followed by a duplicate injection of the test solution and finally of the standard solution. Using this procedure the relative standard deviation (RSD) was 1.30% ($n = 6$; 4 μg ml^{-1}).

Assay of chloroquine in dosage forms

(A) *Ampoules.* Chloroquine from ampoules (50 mg ml^{-1}) was diluted and the internal standard was added such that the final concentration was *ca* 4 μg ml^{-1} (Cq) and exactly 3 μg ml^{-1} (hydroxychloroquine), respectively.

(B) *Tablets.* Ten tablets were weighed and finely powdered; a weight of Cq was taken in water so that the final filtered and diluted solution contained *ca* 4 μg ml^{-1} Cq and exactly 3 μg ml^{-1} hydroxychloroquine.

(C) *Syrups.* Chloroquine syrup (40 mg ml^{-1}) was diluted such that the final concentration contained *ca* 5 μg ml^{-1} chloroquine and exactly 3 μg ml^{-1} hydroxychloroquine.

Results and Discussion

In their study of bioavailability of Cq Mahmoud *et al.* [2] reported no significant differences between the bioavailability of selected brands of Cq tablets and ampoules. On the other hand, Bayoumi *et al.* [3] indicated that *Plasmodium falciparum* had developed resistance to Cq in certain regions of the Sudan, particularly the Eastern areas. If this were to be the case, then an alternative drug would be indicated, since chloroquine would not have the desired therapeutic action against malaria.

The two reports referred to above raise a number of questions. It has been observed that in some patients contracting malaria, the parasite apparently responds differently to the

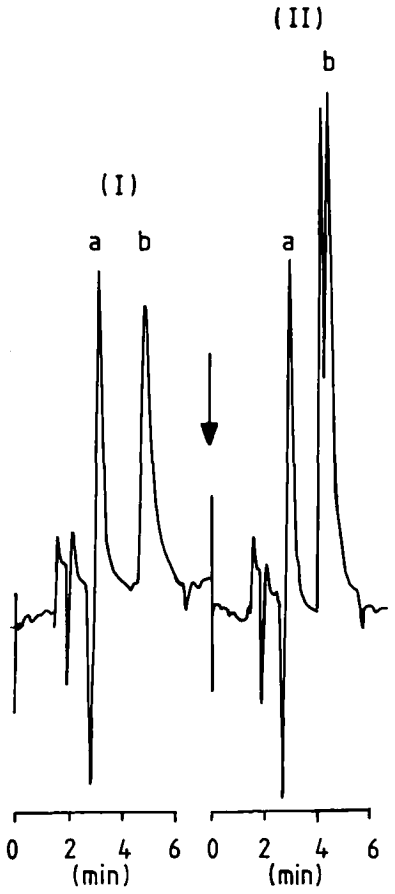

Figure 2
Comparison of (I) standard CQ sample chromatogram with (II) degraded sample of CQ: (a) hydroxychloroquine (internal standard), (b) chloroquine.

different dosage forms supplied by the various manufacturers. Although the colorimetric method of analysis used in one study [3] is sensitive, its specificity for chloroquine is doubtful. In Mahmoud's work [2], the purity of the dosage forms themselves was not examined.

TLC screening of tablets and syrups from each of two manufacturers showed two fluorescent spots for tablets under UV light (the Rf values being 0.23 and 0.60, respectively), while for syrups three spots were found; values 0.2, 0.38 and 0.6. The chloroform extracts of the basified solutions of the two brands of dosage form gave similar results.

It is important to note that in the LC method employed, an apparently single peak (Fig. 2I) was obtained for chloroquine, whereas by TLC chloroquine showed more than one spot. However, it should be noted that the HPLC peaks for extracts from some brands were broad, showing shoulders; indeed, in some experiments, overlapping peaks were observed, as compared with a concurrent standard (Fig. 2II).

Attempts to resolve these peaks using different solvent compositions (in which the content of acetonitrile was varied from 40 to 30%, v/v) failed to completely separate these peaks, indicating that the products have chemical structures similar to that of chloroquine.

Table 1
Content of chloroquinephosphate as % w/w of the label amount in various brands of different dosage forms

	Ampoules		Tablets		Syrups	
Brand*	Mean ± SD		Mean ± SD		Mean ± SD	
A	105	2.0	98	0.3	116	6.0
B	105	0.7	—	—	—	—
C	99.6	2.8	—	—	—	—
D	115	4.5	101	6.1	—	—
E	111	1.0	—	—	—	—
F	129	0.1	—	—	—	—
G	—	—	110	10	112	1.6
H	—	—	109	7.0	—	—
I	—	—	95	3.0	—	—
J	—	—	—	—	115	1.2
K	—	—	—	—	105	5.6
L	—	—	—	—	103	6.6

*Brands or trade name (all as chloroquine phosphate BP, unless otherwise stated).
A, Nivaquine (Chloroquine Sulphate BP) (May and Baker, Dagenham, UK); B, Chloroquine Phosphate BP (Alexandria Pharmaceutical Company, Egypt); C, Chloroquine Phosphate BP (Paris Chemicals, France); D, Resochin (Bayer AG, Germany); E, Delagil (Egis Pharmaceuticals, Budapest, Hungary); F, Chloroquine Phosphate (Lab-Agubant, Paris, France); G, Lagaquin (Lagap S.A., Vezia, Switzerland); H, Malaraquin (Sterling Products International, Khartoum, Sudan); I, Amaquine (Amipharma, Khartoum, Sudan); J, Rivoquine (Rivopharm Laboratories Manno, Switzerland); K, Malarex (Dumex Ltd, Copenhagen, Denmark); L, Scaniquine (Scandrug, Copenhagen, Denmark).

The results obtained for analysis of various brands of the different dosage forms (Table 1) showed that ampoules of brands A, B and C complied with the BP 1980 requirement for chloroquine content (95–105% against label strength). On the other hand, brands D, E and F showed an apparently higher content of chloroquine. It is also clear that tablets from brands A, D and I comply with the BP 1980 required content of chloroquine (92.5–107.5%), while tablets from source G and H were found to contain higher amounts still. Brands A, G and J of chloroquine syrup also showed a higher content of chloroquine. This may be due to overfill by the manufacturer, although the probability of artificially high values of Cq due to coeluting impurities cannot be disregarded at this stage. The Cq content of the various brands of different dosage forms was determined using this method. The results obtained are presented in Table 1.

For a proper evaluation of Cq in its dosage forms, this study shows the importance of developing methods which can reliably separate chloroquine from its decomposition products. Such methods are also important in studies on chloroquine decomposition and are currently being developed in the authors' laboratories. However, these preliminary results indicate the range of variability of Cq samples available in the Sudan.

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