

Evaluation of the physicochemical equivalence of three brands of commercially available quinine sulphate tablets from South Western part of Nigeria

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Abstract

Background: The relatively little resistance to quinine globally has led to an increase in its use in *P. falciparum* malaria especially in multi-drug resistant strains.

Objective: To evaluate the physicochemical and equivalency of three brands of quinine sulphate tablets available in South Western region of Nigeria.

Methods: The pharmaceutical and chemical equivalence of three brands of quinine sulphate tablets were assessed through the evaluation of some biopharmaceutical parameters and active drug content.

Results: All the brands complied with the official specification for uniformity of weight. Two of the brands (A & B) gave similar crushing strengths while the third brand (C) gave a much lower value. Similarly all the brands complied with the official specification of disintegration test but the obtained values were statistically different ($p < 0.05$). The T_{70} obtained from the dissolution rate profile was less than 45 minutes for the three brands, although A and B were not statistically different but C was statistically from A and B. The quinine content of brands B and C are within the official specification however brand A with percentage content of $110 \pm 1.3\% w/w$, is above the specification while it is statistically different from the other brands.

Conclusion: Brands B and C could be regarded as chemical equivalent, but they are not biopharmaceutical equivalents, on the other hand, brands A and B may be regarded as biopharmaceutical equivalents but not chemical equivalent.

Keywords: Quinine sulphate tablets, non-aqueous titration, chemical equivalence, biopharmaceutical equivalence

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Introduction

There have been many reports on the socio-economic burden of malaria in the developing world with the children being the most vulnerable with significant morbidities and mortality¹. The failure of the various malaria control strategies in the recent past makes malaria a topical health and socio-economic issue in the developing world.

The development of drug resistant malaria has necessitated a multi- sectoral approach to the management of malaria infection. This has resulted in the reintroduction of some older antimalarial drugs such as quinine and amodiaquine.

Thus, interest is focusing the reintroduction of quinine as the 1st line of treatment for severe malaria

especially cerebral malaria and also as a second line of treatment for uncomplicated malaria².

Quinine, a quinolone methanol, is the main alkaloid of *Cinchona* species. There is relatively little resistance to quinine worldwide³. It is a laevorotatory stereo isomer of quinidine and it is available in different salt forms which include hydrochloride, sulphate, dihydrochloride, bisulphate etc. Quinine has been used clinically in parenteral treatment of severe malaria and oral treatment of resistant falciparum malaria⁴. Although, decreasing sensitivity to quinine has been detected in areas of South East Asia, the strains of *P. falciparum* from Africa are generally sensitive to quinine^{5,6}.

In line with the WHO recommendation, the federal government of Nigeria adopted the use of Artemisinin-Combined Therapy (ACTs) drugs as first line antimalarial drugs. The ACTs comprises of the combination of artemisinin derivatives with established antimalarial agents such as chloroquine and amodiaquine². Earlier workers have reported in

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vitro and in vivo synergism between artemisinin and quinine^{7,8}. The combination of artemisinin derivatives and quinine have been suggested for multidrug resistance areas where malaria transmission is intensive³.

The choice, quality and time of commencement of anti-malarial drug use along with adequacy of dose of the drugs affect the overall efficacy of antimalarials, thus the quality of antimalarials is important in the roll back malaria programme.

The sourcing of drug product from more than one source has been accompanied by reported cases of variability in clinical responses, which has been attributed to different formulation factors, methods of handling/production, packaging or storage factors as well as outright sub-standard product⁹.

In line with the WHO guidelines, the National Agency for Food, Drug Administration and Control (NAFDAC), which is the drug regulatory authority responsible for the administration and control of drugs in Nigeria, has released standards of quality, efficacy and safety, which are aimed at getting the right quality of drug products to the consumers.

Drug products that are chemically equivalent must be identical in strength, quality, purity while pharmaceutical equivalency is as assessed by similarity in content uniformity, disintegration and dissolution rates¹⁰. In Nigeria, chemical and biopharmaceutical inequivalencies have been reported for some brands of ampicillin and tetracycline capsules as well as ciprofloxacin hydrochloride and metronidazole tablets^{11,12,13,14}. However, in a study on some brands of sulphadoxine-pyrimethamine tablets, chemical as well as biopharmaceutical equivalency was observed with three out of eight brands tested, while the remaining five brands were found not equivalent¹⁵.

A report on the quality of some anti-malarials used in selected African countries revealed a significant problem of sub-standards with percentage failures of active ingredient content ranging from 20% to 67% for chloroquine tablets and 5% to 38% for sulphadoxine-pyrimethamine tablets. Dissolution failures ranged from 5 to 29% for chloroquine tablets and 75% to 100% for sulphadoxine-pyrimethamine tablets¹⁶. Similarly, substandard or fake artemisinin derivatives such as artesunate and dihydroartemisinin have been reported in some parts of South-East Asia with about 38 – 52% reported for artesunate^{17,18}.

In view of the suggestion of the inclusion of the combination of quinine with artemisinin derivatives in the roll back malaria programme, there is the need to evaluate the quality of available quinine preparations in the healthcare delivery system so as not jeopardize the expected therapeutic outcome.

The objective of this study was to evaluate the biopharmaceutical and chemical equivalency of the three brands of quinine sulphate tablets available within the South West part of Nigeria as at the time of this study.

Methods

Three different brands of quinine sulphate tablets from the same batch, with labeled contents of 300mg per tablet were obtained from different retail pharmacies in Ibadan which were representative of the brands available within the South Western part of Nigeria. All the tablets were in blister packs. Quinine sulphate reference pure drug was obtained from BDH Chemical Ltd, Poole England.

Physical Examination of the tablets

All the tablets were white sugar coated with similar shapes, thickness and diameter.

Thin Layer Chromatographic identification

1%w/v pure quinine sulphate and its equivalent solution of tablets prepared in a solvent mixture of chloroform: ethanol (2:1), were identified using thin layer chromatography (TLC). The TLC condition involves the use of Methanol: strong Ammonia solution (100: 1.5) as mobile phase on Silica Gel G₂₅₄ stationary phase with ultraviolet spectroscopy detection at 254 and 365nm¹⁹.

Uniformity of weight determination

Twenty tablets from each of the three brands were weighed individually using Mettler 1180 weighing balance. The average weights of the tablet were calculated as well as their percentage deviation from the average weight. The expected deviation from the mean for the average weight should not be more 5%²⁰.

Hardness Test

The crushing strength of five individual tablets per brand was determined using a Keetan tablet hardness tester.

Tablet Disintegration Test

This was determined at 37°C using Veego disintegration testing apparatus until no particle remained on the basket of the system. The time taking for each of the six tablets tested in each of the brand was recorded.

Dissolution rate determination (B.P. 2001)²⁰

This was determined using the Veego dissolution rate testing apparatus using 0.1M HCl (900 ml) as the dissolution medium. The dissolution medium was maintained at 36.5 – 37.5°C and the basket was rotated at 100 r.p.m. Samples (10ml) were withdrawn at timed intervals of 5minutes for 1hour. 10ml fresh dissolution medium was used to replace the withdrawn samples after each sampling. The samples were filtered and diluted appropriately before the absorbances were measured at 348nm using ultraviolet/visible spectrophotometer (Cecil Instruments Ltd, Cambridge, England). Six tablets were used from each brand.

The content of quinine sulphate in each sample was determined based on the calibration curve obtained with serial dilutions of the pure drug at 5, 10, 20, 30, 40, 50 and 60µg/ml determined at 348nm using pure quinine sulphate powder. The regression equation for the calibration curve was $y = 134.72x + 0.0035$, $r^2 = 0.9948$.

The dissolution profiles of the different brands of quinine sulphate tablets were generated from the graph of the amount of quinine sulphate dissolved versus time. The average T_{70} (time for 70% of the active drug to be dissolved) and the amount dissolved at 45minutes were obtained for each brand.

Chemical content determination

Non- aqueous titrimetry using colour indicator end point was used for chemical content determination of the pure quinine sulphate powder as well as the quinine sulphate tablets (B.P.2001), crystal violet indicator was used as indicator²⁰.

0.05g quinine sulphate powder or its equivalent in the tablet dosage form of the different brands were dissolved in a mixture of 1.8ml chloroform and 3.5ml acetic anhydride and titrated against 0.1M acetous perchloric acid using 0.5%w/v crystal violet solution as indicator until a bluish – green end point. Blank titrations were carried and titre values were adjusted by deducting the blank determination from the assay. All the determinations were carried out in triplicate.

Statistical analysis

Student t-test and one-way ANOVA was used for the statistical analysis, $p < 0.05$ was taken as the significant level.

Result

The TLC analysis of the pure drug and the various brands gave R_f values of 0.50. All the tablets are sugar coated with similar shape and colour although with different inscriptions.

All of the brands complied with the uniformity of weight determinations by not deviating by up to 5% from the mean value (Table 1). The mean crushing strength which is an indication of the hardness of the tablets showed that brands B and C gave similar crushing strengths, while the values obtained for brand A was the lowest (Table 1). The obtained values are statistically different between all the brands ($p < 0.05$).

Table 1: Uniformity of weight, hardness and chemical content determination of three brands of quinine sulphate tablets (Mean ± S.D.)

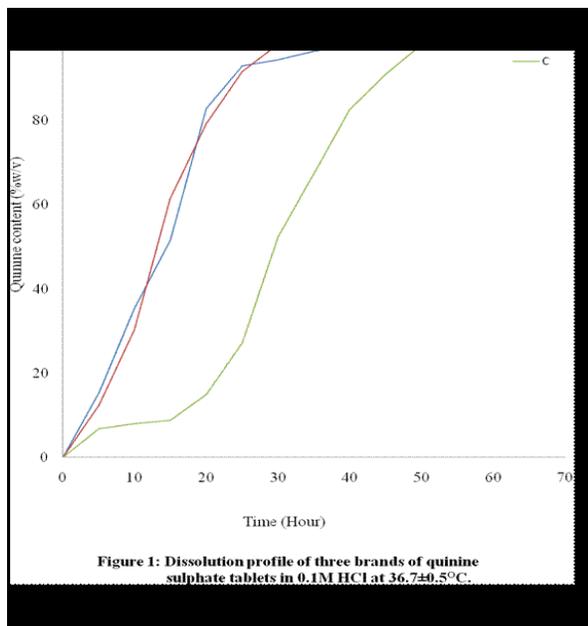
Samples	Uniformity of Weight(mg)	Hardness Test (Mean Crushing strength)(Kg/cm ³)	% Chemical Content(%w/w)
Pure Quinine sulphate powder	-	-	100.2± 4.5
Brand A	673.0±3.6	3.3± 0.45	110.3±1.3
Brand B	626.0± 9.3	6.0± 0.79	98.3± 1.2
Brand C	726.2± 2.6	9.7± 1.89	98.9± 0.75

The disintegration time obtained for all the brands A, B and C were significantly different from each other ($p < 0.05$) (Table 2).

Table 2: Disintegration and Dissolution rate profile for three brands of quinine sulphate tablets at 36±0.5°C

Sample	Disintegration Time(minutes)	Dissolution Rate Profile	
		Time to attain 70% dissolution (T_{70}) (minutes)	% Dissolution at 45 minutes (C_{45})
A	5.6± 0.6	17.6± 2.0	99.3± 1.8
B	11.1± 1.6	16.8± 3.3	101.6± 0.9
C	15.3± 1.2	34.7± 8.1	90.9± 13.5

Figure 1: Dissolution profile of three brands of quinine sulphate tables in 0.1M HCl at 36.7±0.5°C



The obtained dissolution rate profile revealed that all the brands attained more than 70%w/v dissolution by the 45minutes specified in the British Pharmacopoeia, the values were found not to be different statistically with $p>0.05$ (Table 2, Figure 1). However, the time to attain 70%w/v dissolution obtained was similar for brands A and B, while it is statistically different from that of brand C $p<0.05$ (Table 2, Figure 1).

The result of the non-aqueous titration of the pure quinine sulphate powder and the three tablet brands is presented in Table 1. The pure quinine sulphate powder and brands B and C had values within the range specified in the B.P. of 99 – 101%w/v for pure compound and 95 - 105%w/v for the tablet dosage form (Table 1). However, the chemical content obtained for brand A was found to be higher than the official specification (Table 1). The % chemical content obtained for B and C were found to be similar with $p > 0.05$, while brand A was found

to be statistically different from those of B and C ($p<0.05$) (Table 1).

Discussion

All the brands used were within their shelf life at the time of the study. The three different brands of quinine sulphate tablets obtained from different retail pharmacy outlets within Ibadan metropolis were found to be the same as those obtainable within the Southwestern part of Nigeria. The tablets were subjected to a number of tests in order to assess their biopharmaceutical and chemical equivalence. The assessments involved the use of both qualitative and quantitative methods of evaluation. The qualitative methods of evaluation includes tablet description i.e. colour size and shape, which were carried out by visual observation as well as active ingredient identification using thin layer chromatography (TLC). Quantitative evaluations on the other hand involves the used are uniformity of weight, disintegration and dissolution tests as well as chemical content determination.

All the brands are similar in colour, shape and sizes as they are sugar coated white tablet with various identification inscriptions on them. The initial identification procedure using TLC revealed that all the brands contained quinine sulphate as they all gave similar R_f values of 0.50, which compares well with that of the reference pure quinine sulphate.

All the brands complied with the uniformity of weight specification of 5% although brand A gave a deviation of 5.4% which is slightly higher than the 5% official specification but none of the tablet deviated by twice this value (Table 1). This indicates that the weights of the tablets in each batch within each brand are within the expected official specifications.

The mean crushing strength determination is a measure of the degree of hardness of the tablets, brands B and C gave values above the recommended value of 4.0 Kg/cm³, while brand A was lower¹⁰

(Table 1). The obtained values were statistically different from each other ($p < 0.05$). Although, the crushing strength is not an official method of assessing tablet quality, it is still useful in assessing the integrity of tablet dosage forms. However this may not be a problem with this drug as it is usually packed in blister pack.

All the brands passed the disintegration test of less than 60 minutes for coated tablets as specified in the B.P.²⁰ (Table 2). Brand A had the lowest disintegration time. The obtained result seems to be in agreement with the crushing strength obtained for all the brands.

The dissolution rate is a measure of the amount of drug released into the system with time. The dissolution profile of the three brands showed that all the brands met the official specification of 70%w/v dissolution at 45 minutes, (Table 2), however, the rate of dissolution was slower for brand C when compared with the other brands (Figure 1). The slower dissolution rate with brand C may be related to its hardness test and the disintegration time results in which it had the highest values when compared to the other brands.

The dissolution rate of a drug is an important parameter in evaluating the absorption profile of drugs. A drug with poor dissolution profile is regarded as having poor biopharmaceutical characteristics, which may be a direct result of its formulation or method of manufacture. For a number of drugs such as frusemide, mefenamic acid, oxazepam and aspirin tablets, *in vitro* dissolution rate has been found to correlate with *in vivo* results assessed by bioavailability studies^{21,22}. However, a lack of correlation between *in vitro* dissolution rate and *in vivo* bioavailability of one brand of paracetamol has been reported in a study on three brands of paracetamol tablets²³. Thus caution must be exercised in the use of dissolution rate for the assessment of bioequivalence of different drug formulation. The result obtained in this study revealed a faster rate of dissolution with brands A and B, which may indicate a faster onset of action. The time for 70%w/v dissolution (T_{70}) for the two brands were not significantly different from each other ($p > 0.05$), however, the values are significantly different from that of brand C ($p < 0.05$). Furthermore, the percentage dissolved at 45 minutes were similar for brands A and B, while the values are higher than that of brand C, the difference in values were however found not to be statistically significant ($p > 0.05$) (Table 2). All the values obtained are higher than the 70%w/v dissolution specification²⁰, which may

indicate that the overall bioavailability may not be affected by the difference in dissolution profile between the three brands.

Dissolution rate has been reported to have a direct bearing on the bioavailability profile of tablet dosage forms as it can be used to predict the drug release pattern *in vivo*²².

Although comparative bioavailability studies would be required to draw clinical conclusions, the differences obtained in the dissolution profiles may indicate formulation differences that could result in differences in bioavailability.

The chemical content of the different brands were determined using a non- aqueous titration with colour indicator end point determination using 5%w/v crystal violet indicator (B.P. 2001). The pure Quinine sulphate powder as well as brands B and C gave a chemical content which were within the official specification, the values were found to be statistically similar ($p > 0.05$). However, the value obtained for brand A was higher than the official specification and it was statistically different from the other two brands (Table 1). The chemical content obtained for brand A in this study is above the official specification of 95-105%w/w (B.P. 2001). This shows that brand A failed the chemical content specification and could not be regarded as chemical equivalent of the other two brands. Previous studies by Onwujekwe et al (2009)²⁴ reported that 46% of quinine tablets available in the South-East of Nigeria did not meet the USP requirement, while 23.8% and 74% substandard level was reported in Tanzania and Cameroon respectively by other workers^{25,26}.

The obtained result in this study however showed that the non-compliance with official specification of one of the brands is not a problem of being substandard but rather exceeding the specification.

A similar study on the physicochemical equivalence of seven brands of chloroquine phosphate tablets found in the South West of Nigeria reported that two of the seven brands studied contained a level of chloroquine phosphate that exceeded the official specification²⁷. While another report on eight brands of sulphadoxine-pyrimethamine combination reported that one of the brands contained more than the official specification for pyrimethamine¹⁵.

Quinine exhibit dose-related toxicity²⁸, thus the high chemical content of brand A, coupled with the low crushing strength, fast disintegration rate and rapid dissolution profile may have a definite impact

on the bioavailability profile of this brand relative to the other two brands.

This indicates that brand A may not be bioequivalent with the other two brands considering the overall expected bioavailability of all the brands.

Conclusion

Of the three brands evaluated in this study, Brands B and C may be regarded as chemical equivalents although they cannot be regarded as biopharmaceutical equivalents because of the significant differences in their disintegration rates and dissolution profiles.

It was quite interesting to note that none of the brands is manufactured within the country, while only brand C is registered by the country's drug regulating agency (NAFDAC).

The possibility of the interchangeability of the three brands of quinine sulphate tablets can only be verified by evaluating their bioequivalence.

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