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PHYSICOCHEMICAL PROPERTIES OF SOME PAEDIATRIC AND ADULT PRODUCTS OF DIHYDROARTEMISININ-PIPERAQUINE ANTIMALARIAL MARKETED IN NIGERIA

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ABSTRACT

The quality indices of dihydroartemisinin–piperaquine (DP) paediatric and adult (DPP and DPA) formulations of antimalarial were assessed. Moisture content, viscosity, total solid for four (DPP) products along with weight uniformity, tablet hardness, friability, disintegration and dissolution for six (DPA) products, were determined. Assay of chemical content was performed using reverse phase high pressure liquid chromatographic (RP-HPLC) with Zorbax Eclipse XDB C8 column (150 x 4.6 mm, 4.6 µm)), UV detection at 220 nm and flow rate of 0.7 mL/min. Acetonitrile: 10 mM ammonium acetate (70:30%, v/v) and tinidazole served as mobile phase and internal standard, respectively. Statistical analysis of data was performed using Minitab statistical software with one way analysis of variance comparing the parameters among the formulations and confidence interval set at 95%. DPP products showed pH and moisture content within 3.19 to 4.65 units and 2.6 to 4.4%, respectively. Total solid and viscosity values were within 86.2 to 97.2% and 78.5 to 125.8 mPa.s, respectively. DPA products had comparable and satisfactory weight uniformity, hardness, friability and disintegration tests results except DPA5 and DPA6 which failed the weight uniformity tests featuring tablets with deviation from the mean above 5%. All DPP products passed chemical content test with values within 92.65 to 101.22% while DPA2, DPA4 and DPA5 failed. All DPA products showed poor dissolution characteristics with C_{40} values below 60% and T_{70} values above 70 min. All DP products showed varying physicochemical properties that may give differing drug performance *in vivo*.

Key words: Dihydroartemisinin-piperaquine, antimalarial, physicochemical properties, Chemical content, biopharmaceutical indices.

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INTRODUCTION

Malaria ranks high among the causes of morbidity and mortality worldwide[1]. It is a health problem recording over 300 million diagnosis yearly, according to World Health Organization reports[2]. The World Health Organization advocates effective treatment of malaria infection which is premised on the use of efficacious antimalarial agents taken according to an optimized regimen[3].

Resistance to antimalarial agents has been reported severally. Developments of newer agents and recommendations on combination therapy involving the artemisinin derivatives have been particularly effective because they act rapidly and are well tolerated[4]. However, artemisinin- based combination therapy (ACT) drugs have also been birthed with parasite resistance[5, 6]. The battle against parasitic resistance to antimalarial agents is ongoing.

Among the fixed-dose combination (FDC) artemisinin-based combination therapy (ACT) in wide use, dihydroartemisinin-piperaquine (DP) has been reported to give rapid initial reduction in parasite biomass while the sustained effect of the more slowly eliminated partner drug prevents the subsequent recrudescence of the infection[7]. DP has been recommended by WHO since 2010 for the treatment of uncomplicated *falciparum* malaria[5]. The drug offers a promising alternative to other currently available ACTS because of its high efficacy, excellent safety profile, good rating of compliance (once daily dosing scheme) and its prolonged post–treatment prophylaxis[8, 9].

The physiochemical properties of these co-formulated drugs describe the ultimate benefit from the product. Dihydroartemisinin (DHA) is a molecule with intrinsic chemical instability and has been reported unsuitable for use in pharmaceutical formulations[10]. Data have shown that currently marketed DHA preparations failed to meet the internationally accepted stability requirements[10]. At a time when concerted efforts aimed to ban counterfeit and substandard medicine from the malaria market it becomes worrisome that World Health Organization and Public Private Partnership Ventures (PPPV) can support the production and marketing of antimalarial drugs (*i.e*., dihydroartemisinin) with such spurious physiochemical properties[10]. Piperaquine the co-formulated partner is also was used earlier

This present study aimed at assessing the physiochemical parameters bothering on the quality characteristics of adult and paediatric formulations of DP products in the Nigerian market.

MATERIALS AND METHODS

Chemicals

Piperaquine reference powder was kindly donated by Central Research Laboratory of University of Lagos. Methanol, ammonium acetate and acetic acid were of high pressure liquid chromatographic (HPLC) grade. Deionized water was used throughout the study. Generic DP paediatric and adult products were purchased from registered pharmacy in Uyo, Southern Region, Nigeria. Details of drug products are listed in Table 1 and Table 2.

Stock solution preparation

Reference standard piperaquine (10 mg) was accurately weighed and transferred to 10 mL volumetric flask. A volume of 6 mL of the mobile phase consisting of acetonitrile: 10 mM ammonium acetate with 2 mM 1-octanesulfonic acid sodium (70:30%, v/v) were added. The mixture was shaken and sonicated for 10 min to dissolve and thereafter filtered through a 0.45 µm nylon membrane fitter. The filtrate was further diluted to mark.

Table 1: Details of generic DP paediatric products

Product code	Details of products							
	Trade name	Source and batch number	Mf/ Ex date	NAFDAC Reg. number	Colour of powder	Odour / Colour on reconstitution	Taste	Texture
DPP1	P-Alaxin	India PDS010	11/2014 10/2016	Yes	Cream	Metallic /Orange	Bitter	Fine
DPP2	Kinotem Powder	China 150715	07/2015 07/2018	Yes	Pink	Strawberry /cream	Sweet	Coarse
DPP3	Falcidin	Vietnam 13001CX	09/2013 09/2016	Yes	Orange	Brick/Brown	Sweet	Coarse
DPP4	Solartep	China	09/2016	Yes	Orange	Orange	Sweet	Fine

**Mf/Ex date represents manufacturing/Expiry date; NAFDAC Reg. represents National Agency for Food and Drug Administration and Control registration number.*

Table 2: Details of generic DP adult product

Product code	Details of products			
	Trade name	**Source**	**Mf/Ex. Date**	**NAFDAC Registration**
DPA1	Axcin DP	China	09/14 - 09/17	Yes
DPA2	Terocan	India	01/15 - 12/17	Yes
DPA3	Kenmekaxin P	India	12/14 - 12/17	Yes
DPA4	Krosh	China	10/14 - 10/17	Yes
DPA5	Fanmet	China	06/14 - 06/17	Yes
DPA6	D-Artep	China	09/14 - 09/17	Yes

**Mf/Ex date represents manufacturing/Expiry date*

Preparation of internal standard solution (ISS)

Pure reference powder of tinidazole (20 mg) was accurately weighed and transferred into a 0.5 L beaker. This was shaken and dissolved in 100 mL of the mobile phase. The solution was transferred to a 1 L volumetric flask and made up to mark to produce a 0.2 %, w/v solution.

Physicochemical properties

Viscosity

The powder products of DP were reconstituted with 30 mL of distilled water, shaken well to disperse and made up 60 mL mark. The viscosity of the reconstituted products was measured using a viscometer (Mettler Toledo, Germany). A volume of 20 mL of the suspension was placed between the cone and the basal plate at temperature of 32^{o}C with rotation of 5 rpm for 5 mm.

Moisture content

Moisture content of the DPP formulations were determined using 1 g of powder in a moisture analyzer (PCE-MB 210C, UK) and heating up to a temperature of 105^{o}C. The moisture content determination was performed in triplicate.

Determination of pH

The probe of pH meter (Mettler Toledo, Germany) was dipped into a 100 mL beaker containing 50 mL of the reconstituted product at temperature of 25^{0} C and the pH determined. This was performed in triplicate.

Total solid

A volume of 20 mL of reconstituted powder was sampled using a pipette from the same depth and (n=3) dispensed into porcelain dishes of known weight (W1). The dishes were placed on water bath to allow the content evaporate to dryness. These were afterward placed in an oven Gallenkamp No. 335 (England). The dishes were removed and allowed to cool intermittently while weighing, till a constant weight was observed, (W2). The difference in weight (W_1-W_2) was obtained and the total solid percent determined. This was performed in triplicate.

Weight uniformity test

Twenty tablets from each of the 6 brands of DP generic were randomly sampled and weighed using a digital weighing balance (Adventure Ohaus, China).The mean weight and percent deviation of each tablet weight from the mean were determined.

Friability test

Ten tablets from each of the six brands of DP tablets were randomly sampled. The total weights of tablets for each brand were recorded (W1).The tablets for each brand was transferred in turns into tablet Friabilator (Roche, UK). The final weights for each brand were determined after 100 cycles of tumblings of the tablets, (W2). The difference in weight (W1-W2) was determined and the percentage weight loss calculated from Equation 1.

$$\%\ Friability = \frac{W1-W2}{W1} \times 100 \ldots (1)$$

Disintegration Test

A tablet each was placed in the basket disc of digital tablet disintegration tester, (TF – 2D, UK). The tester was operated at 37^{o}C while the time for complete disintegration of tablet (with no palpable remains of the tablet on the basket).

Hardness Test

Ten tablets from each brand were randomly selected and each tablet placed between the jaws of hardness tester (Mosanto, England). The force (KgF) required to break the tablets were determined.

Dissolution Test

The dissolution profiles for the DP brands were determined using 500 mL of 0.1 N hydrochloric acid as dissolution media in a dissolution apparatus model RCZ-6C3, (China,). The system was maintained at 37^{o}C and stirring speed of 100 rpm. A volume of 5 mL was sampled at 0, 5, 15, 20, 30, 60 and 15 min while

same amount of fresh medium replaced after each sampling.

Chemical content determination

Preparation of graded concentrations

Graded concentrations of piperaquine were prepared from the stock solution using the dilution formula. The ISS was spiked into the graded concentrations at a final concentration of 20 µg/mL and ready for injection. The graded concentrations were used to prepare the calibration curves.

Preparation of samples from products

Three randomly selected samples from the DPP products were obtained. An equivalent weight of 50 mg piperaquine was accurately taken from each of the containers. Similarly, a total of 10 tablets from each of the DP adult products were randomly selected and weighed together. The average weight was calculated. The tablets were crushed and triturated together. Equivalent weights of 50 mg piperaquine were calculated and accurately weighed for each of the products. This was dissolved in 100 mL of mobile phase by shaking and sonication. The solutions were filtered using 0.45 µm acrodysc syringe filter. The resulting solutions were further diluted to produce a 30 µg/mL working concentration. The final concentration was spiked with ISS to produce 20 µg/mL. The amount of piperaquine was determined using HPLC system

Statistical Analysis

Data obtained were analyzed with Minitab statistical package (Minitab Inc., USA), using one way analysis of variance (ANOVA) and significant difference between values among products indicated at $p < 0.05$.

RESULT AND DISCUSSION

This study assesses the physicochemical properties of DP products after they have been released to the market (*i.e*., within their shelf life). Marketing of adulterated and substandard antimalarial drugs have been reported in the study area[11]. Speculations of drug product instability are also associated with the dihydroartemisinin content of this particular co-formulated product.

The physicochemical properties that relate to quality have been investigated for the paediatric products (*i.e*., pH, moisture content, total solid and viscosity. The adult formulation has been evaluated and quality assessment based on uniformity of weight determination, tablet hardness test, friability and disintegration test. Several reports have presented the unreliability of dihydroartemisinin level quantization in DP formulations [12] while some other workers have also presented some validated methods for determination of dihydroartemisinin alone[13] and simultaneous determination of both drugs in DP[14, 15]. In this study, the piperaquine component has been followed up for the quality assessment of the DP products.

Table 3 presents the results of the physicochemical properties analyses for the paediatric products of DP. The outcome of pH for the products gave a significantly higher value for DPP1 compared with the other investigated products, $p < 0.05$. The levels of pH in products affect the chemical stability of the drug or drug product. In cases where pH varied significantly, it is expected that the chemical content be affected accordingly. In the same vein, the amount of moisture in the powdered product plays a significant role in the chemical stability, crystal structure, powder flow and in the dissolution while reconstitution for use. Products with high moisture content have been observed with aggregation of tablets and caking. Among the products, DPP3 had a high moisture content percent that may cause decomposition of susceptible active ingredient. Products with high moisture content have characteristically poor dissolution profile. All the products have higher than expected moisture content percent that gives an indication that the actives or excipients have absorptive tendencies. Manufacturers of paediatric DP products may therefore need to present formulations with lower moisture content powder with better free flowing character.

Table 3: Physicochemical properties of DP powder for paediatric suspension

Product code	Physicochemical parameters (n=3)			
	pH	Moisture content (%)	Total solid (%)	Viscosity (mPa.s)
DPP1	4.65.±.0.02	3.9 ± 0.12	91.3.±.0.7	78.5 ± 6.9
DPP2	3.52 ±.0.01	2.6 ± 0.06	97.2.±.1.4	125.8.±.3.7
DPP3	3.56 ± 0.05	4.4 ± 0.02	86.2 ± 3.7	85.8 ± 3.5
DPP4	3.19 ± 0.03	4.1 ± 0.04	94.8 ± 5.8	98.3 ± 1.4

**mPas.s represents millipascal-second*

Product DPP2 presents with significantly higher viscosity compared to the other products, $p < 0.05$. The viscosity observed for DPP2 may be a function of the excipients employed in the formulation. The choice of excipients to improve the thickness of powders for reconstitution however should be carefully selected to allow the timely release of the actives from the formulation[18]. Products with high viscosity are known to have low release rates of the active ingredients from the tablet matrix. There was no significant difference in the total solid percent of the sampled DPP products, $p > 0.05$.

The maximum deviations from the mean of tablet weights for the selected DP products were within the pharmacopoeia range of not more than 5% except product DPA5 and DPA6 (Table 4). None of the tablets in these products had twice as much deviation. Only one of the tablets in the two groups had this deviation. Hardness test for all the products lay within 3.24 to 8.60 kgF. These values are acceptable range for conventional immediate release tablets (Table 4). Values below 3.0 are common with chewable tablets[16, 17]. Friability values for the products lay within 0.111 to 0.343 therefore considered to have satisfactory indices. Evaluation of the disintegration time for the tablets also gave satisfactory values (1.45 to 17.82 min). The general comment on these paediatric products therefore is that the manufacturing processes by the various sources have strived to achieve quality products by adhering to standard operating procedures.

Table 4: Physical parameters for DPA products

Product code	Some physical parameters of DP adult products			
	Maximum deviation from mean weight (%)	Hardness test (kgF)	Friability	Disintegration time (min)
DPA1	1.708	5.99	0.111	11.60
DPA2	2.877	6.82	0.116	1.45
DPA3	3.600	5.34	0.140	5.82
DPA4	2.700	8.60	0.125	10.44
DPA5	5.061	3.24	0.343	17.82
DPA6	6.694	6.49	0.141	1.78

The coefficient of regression (R^2) for the calibration plot for the determination of piperaquine in the drug products was 0.997. Piperaquine chemical contents for the various DP products varied significantly, $p<0.05$. Table 5 presents the mean piperaquine contents and their respective standard deviation values. It was observed that the values for the paediatric products lay within the International Pharmacopoeia specification of between 90% and 110% while some of the adult products (DPA2, DPA4 and DPA5) fell out of the range with values above 110%[19].

Table 5: Chemical content of paediatric and adult products of DP

Product code	**Chemical content**	**Comment**
DPP1	98.23 ± 5.65	S
DPP2	92.65 ± 3.14	S
DPP3	101.22 ± 4.65	S
DPP4	96.48 ± 8.62	S
DPA1	107.53 ± 2.85	S
DPA2	125.0 ± 7.73	NS
DPA3	93.50 ± 7.40	S
DPA4	131.47 ± 8.84	NS
DPA5	114.33 ± 8.20	NS
DPA6	104.33 ± 9.64	S

*S and NS represent satisfactory and not satisfactory values with respect to International Pharmacopoeia specifications for actives in drug products.

The selected products had variable dissolution profile in 0.1 N hydrochloric acid. Figure 1 expresses the dissolution chart of the six DPA products. The dissolution parameters are laid out in Table 6.

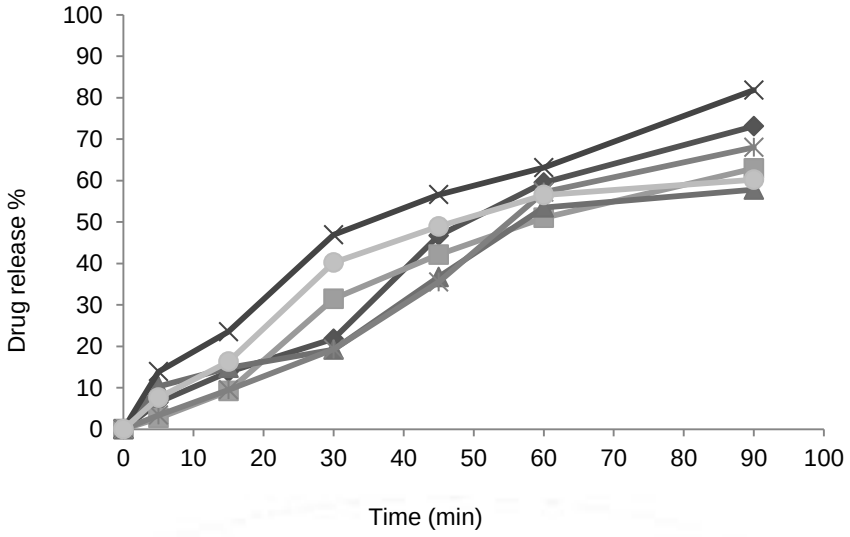

Figure 1: Dissolution profile for DP tablets following piperaquine release DPA1♦, DPA2▄, DPA3▲, DPA4×, DPA5* and DPA6•)

Dissolution tests specification according to USP 2014 states that each unit should have not less than Q+5% of the active ingredient dissolved in 60 min for the six units or tablets. If the requirement is not met, another six units should be tested and the mean percent dissolution for the twelve units should be less than Q% and none of the units should be less than Q-15 %. It was necessary to compare the percentage dissolved statistically so as to compare the various DPA products in circulation for their pharmaceutical equivalence[200].

The area under the curve (AUC) for each product was calculated as

$$AUC = \sum_{i=1}^{i=n} \frac{(t1-ti-1)(y2-1+yi)}{2} \ldots(2)$$

"ti' is the 'ith' time point and 'yi' the percentage of dissolved product at time 'ti'. The AUC of the products relates to their rate and extent of drug release and in many drugs may correlate with the bioavailability. The AUC for the adult products showed statistically significant difference among the products p>0.05. None of the DPA products had satisfactory dissolution profile with respect to piperaquine release. The amount released at 40 min (C_{40}) for the products vary widely and in the range (30.0 to 46.7%). DPA1, DPA2, DPA4 and DPA5 did not achieve 70% dissolution within the experimental time of 90 min.

Table 6 presents the dissolution profile of the generic DPA in the study. Dissolution efficiency was calculated as

$$DE = \frac{\int_{t1}^{t2} y.dt}{y100\times(t2-t1)} \times 100 \ldots(3)$$

Table 6: Dissolution parameters for DPA products

Product code	Dissolution parameters				
	C_{40} (%)	**T_{70}** Min	**AUC** %.min	**DE**	
DPA1	46.7 ± 2.54	**	3684.0	1.0	
DPA2	40.0 ± 1.79	**	3329.35	0.90	
DPA3	33.0 ± 0.45	70.0 ± 2.55	3042.88	0.83	
DPA4	56.0 ± 5.12	**	4560.60	1.24	
DPA5	40.0 ± 2.86	**	3273.25	0.89	
DPA6	30.0 ± 3.65	85.0 ± 4.90	3771.50	1.02	

**C_{40}, T_{70}, AUC, DE, represent the amount of drug released at 40 min, time to achieve 70% dissolution, area under the concentration time curve, dissolution efficiency for the drug products.*

***Values outside of dissolution experimental time.*

CONCLUSION

The sampled DP products exhibited varying physicochemical properties that may present different drug performance. The quality characteristics of the products also varied significantly among the products. There is the need for a standard operating procedure for the manufacture of DP paediatric and adult products for bioequivalence considerations.

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