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Quality Assessment of Twenty Brands of Sulfadoxine-Pyrimethamine tablets marketed in Mainland area of Lagos

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Abstract

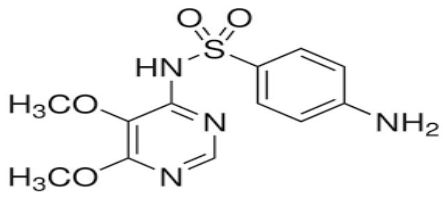
Globally, malaria remains the most important health care problem and is estimated to be responsible directly for about 3000 deaths a day worldwide. Sulphadoxine-Pyrimethamine (SP) is a combination therapy for serious malaria infections especially in areas where other medicines may not work. Due to the inhibition effects of the combination drugs at 2 different steps in the biosynthesis of tetrahydrofolate, the administration of SP results in a synergistic action against susceptible plasmodia. The low quality of many antimalarial formulations may be responsible for the resistance posed by Plasmodium strains against the drug. This study was focused at investigation of physicochemical parameters of 20 brands of SP tablets sampled from reputable pharmacy stores within the mainland area of Lagos, Nigeria. Physicochemical tests were conducted according to standard procedures specified in pharmacopoeia monographs, while dissolution and chemical assay were carried out using dissolution apparatus USP 2 and samples were analysed with UV-spectrophotometer and HPLC respectively. Results show no major discrepancies observed for uniformity of shape, size, color and all the brands assayed passed the visual inspection tests for authenticity of packaging materials. Hardness ranged from 5.76-8.74 kg/cm^2, friability from 0.18-1.60 %, while the mean disintegration times ranged from 4.3-17.7 minutes and 95% of the brands conformed to official specified values for the physicochemical parameters. In this study, all the brands assayed conform to official USP 2013 purity range of 90-110% and passed the chemical assay for pyrimethamine, while 70% of the brands passed the chemical assay for sulphadoxine content. In this study, sulphadoxine generally gave a better release than pyrimethamine under the dissolution conditions, while 20% of brands assayed with mean release ranging from 50.9-58.7% at 30 minutes failed the dissolution tests for both APIs, 80% of the brands, having released ≥ 60% of the labeled amount of drug at 30 minutes, complied with the standard dissolution rates for active pharmaceutical ingredients. The result of this study indicates that the registration process and post-market surveillance in the country is having a significant impact as none of the brand assayed could be considered of very poor quality.

Keywords: Pyrimethamine, sulphadoxine, physicochemical, dissolution, spectrophotometer

Introduction

Globally, malaria remains the most important health care problem and is estimated to be responsible directly for about 3000 deaths a day worldwide (1). The disease is transmitted by infected female Anopheles mosquito, which bites and introduces the parasites from saliva into a person's blood, while the parasites then travel to the liver where they mature and reproduce (2). Malaria presents symptoms like fatigue, fever, vomiting and headaches, but in severe cases, can cause seizures, coma or death. *Plasmodium vivax*, *P. ovale* and *P. malariae* generally cause a milder form of malaria, while most deaths are caused by *Plasmodium falciparum* (3). Along with preventive measures, several medications are available for malaria treatment and antimalarials are among the most widely consumed drugs in most tropical

countries including Nigeria (4). Occasional doses of SP are recommended as a combination drug in treatment and prevention of malaria caused by chloroquine-resistant strains of *Plasmodium falciparum* and also during the first trimester of pregnancy (5). The combination can also be taken for treatment of isosporiasis, however it may cause some serious side effects and hence, it is often used to treat serious malaria infections in areas where other medicines may not work (6, 7). Each tablet contains 500mg N^1–(5, 6-dimethoxy-4-pyrimidinyl) sulfanilamide (sulfadoxine) and 25mg 2, 4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (pyrimethamine) (8). The sulphadoxine (figure 1) are bacteriostatic antimicrobials that inhibits dihydropteroate synthetase, hence blocking the incorporation of p-aminobenzoic acid to form dihydropteroic acid, while pyrimethamine (figure 1) are antiprotozoal agent which selectively inhibits dihydrofolate reductase and interferes with tetrahydrofolic acid synthesis in most species of plasmodium (9). With the emergence of several generic brands of SP, the World Health Organization (WHO) has provided guidelines for registration and quality control of pharmaceutical products to ensure their compliance with the quality and safety as innovator brands. Several authors have investigated the physicochemical and bioequivalence parameters of some drug products marketed in Nigeria (10 - 13) and in some countries (14 - 16). The commonly employed detectors for pharmaceutical compounds and metabolites include spectrophotometric, electrochemical and fluorescent (17, 18). To verify compliance with the regulatory standard, current study was focused at investigating the physicochemical parameters, in-vitro dissolution and chemical assay of 20 brands of SP tablets sampled from pharmacy stores within the mainland area of Lagos, Nigeria. Also the level of substandard SP tablets will be ascertained.



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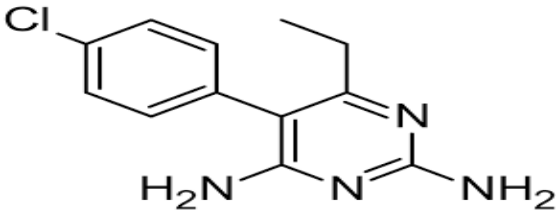

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Figure 1: Structure of Sulphadoxine (1) and Pyrimethamine (2)

Materials and methods:

Reagent and Apparatus

All reagents were of analytical grade and were freshly prepared. Pyrimethamine, sulphadoxine and metronidazole reference standards, potassium dihydrogen orthophosphate, sodium hydroxide pellet (99-101%), HPLC grade methanol and acetonitrile were all obtained from Merc chemicals (Darmstadt Germany). An Agilent Technologies, U.SA 1200 series HPLC system with a C-8 (Zorbax Eclipse XDB RP C8 150x4.6mm, 5µm particle size) column was used in the chemical analysis, syringe filter 0.45 µm millipore®), Mettler Toledo® AL 204 analytical balance, Jenway® pH meter, micropippete, Clifton ultrasonicator, dissolution apparatus USP 2 (COPLEY NE6-COPD dissolution tester), Erweka hardness tester®, Erweka friabilator and multiunit disintegration tester electrolab® apparatus were employed in the study.

Determination of physicochemical parameters

The 20 brands of SP tablets were purchased from reputable pharmacy stores within mainland area of Lagos and information on visual inspection (country of origin, tablet color, shapes, cracks, brand names, batch number, pharmacopeia status, manufacturing and expiry dates) were recorded and brands were randomly coded (ASP 1-20). None of the drugs had expired as at the time of conduct of this study. The physicochemical parameters were determined as specified in British pharmacopoeia (19). The 20 tablets for weight uniformity tests were randomly selected from each brand and mean weight, standard deviation (SD) and % SD were determined. For hardness test, each tablet was placed between the spindles of tester, the knurled knot was adjusted to hold the tablet in position and applied pressure gradually increased until the tablets broke and the pressure applied at break point was recorded. For friability test, each pre-weighed tablets from the brands were placed in automated friabilator which was operated at a speed of 25 rpm for 4 minutes, tablets were re-weighed and loss in weight determined as a % of initial weight. The disintegration times of 6 tablets from each brand were determined in distilled water at 37 ± 0.5^{o}C and all determinations were in triplicates.

HPLC analysis

Preparation of reference standards

A 100 mg and 5 mg of sulfadoxine and pyrimethamine reference standards were weighed respectively into separate 100 ml volumetric flask containing 60 ml mixture of diluents (acetonitrile: water, 1:1) and sonicated to dissolve. This was made up to 100ml mark to obtain concentrations of 1000 and 5 µg/ml. The following concentrations of mix standard of sulfadoxine (25, 37.5, 50, 75, 100, 150 and 200µg/ml) and pyrimethamine (1.25, 1.875, 2.5, 3.75, 5.0, 7.5 and 10 µg/ml) were each prepared from the stock for calibration curve. A 25.0 µl of 1.0 mg/ml metronidazole internal standard (IS) was spiked into each solution to obtain the final concentrations.

Sample preparation

Twenty tablets of each sample were randomly un-blistered, weighed and triturated into fine powder. Average weights were determined, equivalent of 100 mg and 5 mg of sulphadoxine and pyrimethamine were weighed respectively into 100ml volumetric flask containing 60ml of diluents. This was sonicated to dissolve and make up to mark with diluents to obtain 1000 and 5µg/ml of sulphadoxine and pyrimethamine respectively, from which working concentrations of 75 and 3.75µg/ml was prepared and filtered. This was similarly spiked with metronidazole (IS) when obtaining the final concentrations.

Chromatographic conditions

The chromatographic conditions were according to modified method (20). The mobile phase consists of 40% acetonitrile and 60% of 25mM KH_2PO_4 buffer while pH of the solution was adjusted to 3.7 using orthophosphoric acid and at a flow rate of 0.8 ml/min. HPLC separation was by using a reverse phase C8 column (150x4.6mm, 5µm particle size) at ambient temperature, while the chromatographic signal was monitored with variable wavelength UV detector at 229 nm.

In –vitro dissolution studies

In vitro dissolution studies were according to the method (21). The calibration curves and regression equations were used to determine the concentration dissolved per time using absorbances obtained at 288 nm and 220 nm for pyrimethamine and sulfadoxine respectively.

Quality assurance

All glasswares were thoroughly cleaned to avoid contamination and ensure method reliability. The calibrated equipments and validated standard operating procedures were employed. All the test samples were within their shelf life at the time of investigation and solutions were filtered to remove particles which may block the column. The mean weight, standard deviation (SD) and % SD were calculated for each brand and % released during dissolution was determined using the calibration plot from UV spectrophotometric analysis. For HPLC analysis, the peak area ratio (PAR) of reference standard (and samples) to internal standard was calculated. The calibration plot and regression

equation were obtained using the Microsoft excel 2007. The experimental concentrations were obtained by substitution of the mean PAR of the test sample into the regression equation, while theoretical concentrations were the prepared 75 and 3.75µg/mL for sulfadoxine and pyrimethamine respectively for the test samples. The purity was calculated as the ratio of experimental to theoretical concentrations expressed as a %. The APIs in test sample was deemed acceptable, if the purity at 95% confidence interval lies within 90-110% (22).

Result and Discussion

In this study, all the brands assayed (Table 1) conformed to standard specification for uniformity of weight test (Table 2).

Table 1: Sample information

Brand code	Manufacturing Date	Expiry Date	NAFDAC approval	Country Of Origin	Colour of Tablet	Pharmacopeia Status
ASP 1	FEB 2014	JAN 2018	Yes	INDIA	WHITE	USP
ASP 2	MAR 2014	FEB 2017	Yes	INDIA	WHITE	USP
ASP 3	MAR 2014	FEB 2017	Yes	INDIA	WHITE	USP
ASP 4	APR 2014	MAR 2017	Yes	INDIA	WHITE	USP
ASP 5	FEB 2015	JAN 2018	Yes	NIGERIA	WHITE	USP
ASP 6	JULY 2014	JUNE 2017	Yes	INDIA	ORANGE	USP
ASP 7	OCT 2014	OCT 2017	Yes	CHINA	WHITE	USP
ASP 8	JULY 2014	JUNE 2017	Yes	NIGERIA	WHITE	USP
ASP 9	SEP 2013	AUG 2016	Yes	NIGERIA	WHITE	USP
ASP 10	JAN 2014	DEC 2016	Yes	INDIA	WHITE	USP

Table 2: Result of weight uniformity test for SP brands

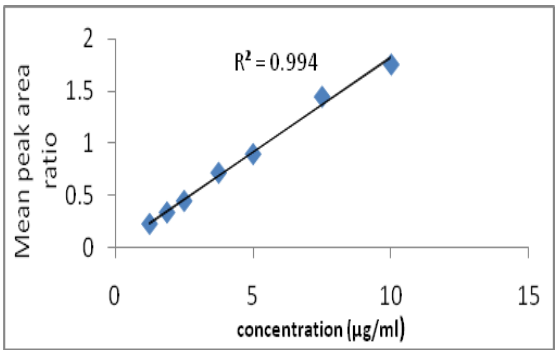
Weight of individual tablet (g)										
Tablet No	ASP1	ASP2	ASP3	ASP4	ASP5	ASP6	ASP7	ASP8	ASP9	ASP10
Sum	13.1388	12.0121	12.0241	12.8533	12.0939	12.8127	12.8696	11.661	11.6399	12.2135
Mean	0.6569	0.6006	0.6012	0.6427	0.6047	0.6406	0.6435	0.5831	0.5820	0.6107
SD	0.0071	0.0052	0.0070	0.0050	0.0052	0.0051	0.0060	0.0248	0.0115	0.0139
% SD	0.7117	0.5237	0.7003	0.5042	0.5178	0.5129	0.5950	2.4783	1.1467	1.3889

The significance of weight uniformity test was to ensure that the tablets in each batch were within the appropriate size range and uniformity of weight was considered permissible, if % SD in weight for tablet ≤ 250 mg does not exceed ±5% (19).

Table 3: Results of hardness, friability, disintegration tests and HPLC chemical assay of SP brands

Brand code	Hardness (kg/cm^2)	Friability (% loss)	Disintegration time (s)	% of Sulfadoxine	% of Pyrimethamine
ASP 1	6.95	0.69	500.80	90.44	102.57
ASP 2	5.78	1.60	581.60	91.19	98.17
ASP 3	6.89	0.43	491.90	90.45	105.49
ASP 4	7.12	0.95	257.70	76.74	95.24
ASP 5	7.43	0.42	764.80	90.56	91.80
ASP 6	6.72	0.65	890.70	79.70	98.17
ASP 7	6.68	0.68	199.10	91.56	98.17
ASP 8	7.90	0.18	698.20	82.07	96.70
ASP 9	6.78	0.42	658.30	90.89	96.70
ASP 10	8.00	0.67	325.40	90.47	95.60
ASP 11	5.83	0.79	580.50	85.14	98.50
ASP 12	6.74	0.65	681.30	90.20	97.60
ASP 13	5.76	0.76	691.90	90.54	101.49
ASP 14	7.35	0.95	157.50	93.42	97.80
ASP 15	6.43	0.42	764.60	78.58	102.30
ASP 16	5.86	0.65	720.70	90.64	98.45
ASP 17	6.43	0.68	399.10	84.65	100.50
ASP 18	8.74	0.68	1060.20	91.45	98.75
ASP 19	5.96	0.76	756.30	90.27	101.70
ASP 20	7.08	0.18	828.40	92.56	95.60

The hardness value ranged from 5.76-8.74 kg/cm^2 and 95 % of the brands assayed lies within the official acceptable value of 5-8 kg/cm^2, friability ranges from 0.18 to 1.60 %, while 95 % of the brands conformed to the acceptable limit of loss ≤ 1% for compressed tablets (23). The mean disintegration times ranged from 4.3-17.7 minutes and 95% of the brands conformed to specified official standard that an uncoated tablet is expected to disintegrate within 15 minutes (21). Hardness gives an indication of the mechanical integrity of the tablet and could be a major factor influencing disintegration. In addition, if a tablet is too soft, it may not be able to withstand the handling during the process of coating and packaging. The friability test gives evaluation of the ability of the tablet to withstand abrasion during packaging, handling and shipping, while dissolution is a measure of mean concentrations of APIs released into the medium with time. Disintegration is a necessary condition for dissolution and could be the rate-determining step during drug absorption. Some of the major factors influencing physicochemical parameters include the amount and nature of binders, moisture content during compression, force of compression during the binding as well as method of granulation of tablets (10). The analytical parameters are as shown in Table 4, while the calibration curves are as shown in figure 2. The regression coefficient (R^2) from the equations (y=0.0384x+0.1211; y= 0.0914 x - 0.0585) of the analytical parameters varied from 0.994-0.999.



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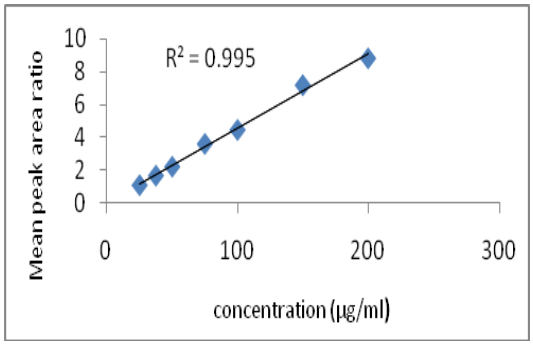

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Figure 2: HPLC calibration plot for pyrimethamine (1) and sulphadoxine (2)

Table 4: Analytical parameters

Analyte	**Pyrimethamine**		**Sulphadoxine**	
Molecular weight	248.71		310.33	
Analysis	UV	HPLC/UV	UV	HPLC /UV
Retention time	-	2.38	-	3.34

All the 20 brands assayed passed the chemical assay for pyrimethamine, having complied with the official purity range of 90-110% (20), while 14 brands passed the chemical assay for sulphadoxine content (table 3). The pyrimethamine in the combination drugs is antiprotozoal agent which selectively inhibits dihydrofolate reductase (24) and interferes with

tetrahydrofolic acid (THFA). The THFA is required for DNA and RNA synthesis in most species of plasmodium. The pharmacopoeia monograph specifies that for both APIs, the amount of drug released after 30 minutes during dissolution experiment should be ≥ 60% of the labeled amount (22).

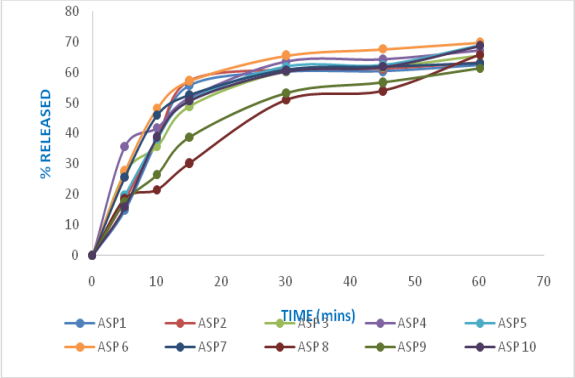

Figure 3**: %** of pyrimethamine release from brands of SP tablets in phosphate buffer, pH 6.8

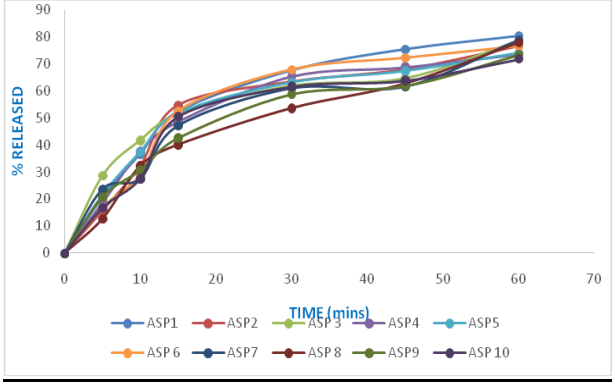

Figure 4**: %** of sulphadoxine release from brands of SP tablets in phosphate buffer, pH 6.

In this study, 8 of the 10 Brands (ASP 1-10) analyzed complied (figure 3 and 4) with the standard dissolution rates for both active ingredients, while 2 Brands (ASP 8 and 9) with mean dissolution ranging from 50.9-58.7% in 30 minutes failed the dissolution tests for both APIs. Generally, sulphadoxine gave a better release than pyrimethamine under the dissolution condition.

Conclusion

The bioavailability and systemic activity of any solid dosage drug formulation is widely determined by a number of variables including uniformity of weight, hardness, disintegration, friability and chemical assay for drug components, with increasing number of generic brands in the country, there is need to constantly evaluate the aforementioned parameters to ascertain therapeutic viability of the drug tablet formulation and to trounce substandard drugs in Nigeria.

References

1. C. Hughes, R. Tucker, B. Bannister (2003). Malaria prophylaxis for long term travelers. *Communicable Disease and Public Health*; 6 (3):200-208.
2. R.A. Esther, F.A. Brandon, D.R. Christiaan, M. Irma, H.B. Jos, H.M.S. Jan (2003). An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. *Toxicology and applied pharmacology* 189: 233-246.
3. M.D. Green (2006). Antimalarial drug resistance and the importance of drug quality monitoring. *American Journal Tropical Medicine and Hygiene*, 78(4), 2008: 552-555.
4. K. A Hall, P.N. Newton, Green M.D, M. DeViej, P. Vandenabeele, D. Pizzanelli, M. Mayxay, A. Dondorp, F.M Fernandez (2006). Characterization of counterfeit artesunate antimalarial tablets from southeast Asia. *American Journal of Tropical Medicine and Hygiene*, 75: 804-811.
5. D. Schurmann, F. Bergmann, H. Albrecht, J. Padberg, T. Grunewald, M. Behnsch, M. Grobusch, M. Vallee, T. Wunsche, B. Ruf, N. Suttorp (2001). Twice weekly Pyrimethamine-Sulfadoxine effectively prevents

pneumocystis carinii pneumonia relapse and toxoplasmic encephalitis in patients with AIDS. *The Journal of infection*; 42(1):8-15.

6. D.J. Lange, P.M. Anderson, R. Remanan, S, Marklund, D. Benjamin (2013). Pyrimethamine decreases level of SOD 1 in leucocytes and cerebrospinal fluid of ALS patients: a phase 1 pilot study. *Amyotrophic lateral sclerosis and frontotemporal degeneration*; 14(3):199-204.

7. D.O. Freedman (2008). Clinical practice; malaria prevention in short-term travelers. *New England Journal of medicine*; 359 (6):603-612.

8. R. Hamilton (2015). Tarascon Pocket Pharmacopoeia 2015 Deluxe Lab-Coat Edition. Jones & Bartlett Learning. p. 54

9. A.S. Limpert, M.E. Mattmann, N.D. Cosford, (2013). Recent progress in the discovery of small molecules for the treatment of amyotrophic lateral sclerosis (ALS). *Beilstein Journal of organic chemistry*; 9:717-732.

10. M.A. Odeniyi, O.A. Adegoke, R.B. Adereti, O.A. Odeku, O.A. Itiola (2003). Comparative analysis of 8 brands of sulfadoxine-pyrimethamine tablets. *Trop Journal of pharmaceutical research*, 2:161-167.

11. O.A. Bamiro, M.A. Odeniyi, O.A. Osonuga (2007). Brand variations in the physicochemical properties of metronidazole tablets. *Nigerian Quarterly Journal of Hospital Medicine*, 17: 22-25.

12. O.A. Adegbolagun, O.A. Olalade, S.E. Osumah (2007). Comparative evaluation of the biopharmaceutical and chemical equivalence of some commercially available brands of ciprofloxacin hydrochloride tablets. *Tropical Journal of Pharmaceutical Research*, 6: 737-745.

13. C.O. Esimone, F.B. Okoye, B.U. Onah, C.S. Nworu, E.O. Omeje (2008). In vitro bioequivalence study of nine brands of artesunate tablets marketed in Nigerian, *Journal of Vector Borne Disease*, 45: 60-65.

14. O.M. Minzi, M.J. Moshi, D Hipolite, A.Y. Massele, G. Tomson, O. Ericsson, L.L Gustafsson (2003). Evaluation of the quality of amodiaquine and sulphadoxine/pyrimethamine tablets sold by private wholesale pharmacies in Dar Es Salaam, Tanzania. *Journal of Clinical Pharmacy and Therapeutics*, 28:117-122

15. A.A. Amin, R.W. Snow, G.O Kokwaro (2005). The quality of sulfadoxine-pyrimethamine and amodiaquine in the Kenyan retail sector. *Journal of Clinical Pharmaceutical Therapeutics*. 30:559–565.

16. H.K Chepkwony, N. Mwaura, E. Guantai, E. Gathoni, F.N. Kamau, E. Mbae, G. Wanganga, S. Muteru, N. Birgen, M. Wandeto (2007). Quality of antimalarial drugs analysed in the national quality control laboratory during the period, 2002-2005. *East Central African Journal of Pharmaceutical Science*, 10:59-62.

17. R. Canaparo, E. Muntoni, G.P. Zara, C. Dellapepa, E. Berno, M. Costa, M. Eandi (2000) Determination of ibuprofen in human plasma by high-performance liquid chromatography: validation and application in pharmacokinetic study. *Biomedical Chromatography*, 14(4):219–226.

18. P.S. Gong, S.L. Jeng, Y.F Hsu, C.C Lin, S.Y Lin (2002). Research note: development of a method for the determination of pyrimethamine concentrations in feeds by ion-pairing high-performance liquid chromatography. *Journal of Food Protection*, 65(4):688–691

19. B.P. British Pharmacopoeia (2012). The Pharmaceutical Press, Her Majesty's Stationery Office, for the Department of Health on behalf of the Health Ministers, London.

20. R. Kirim, K. Mustapha, C. Isimi, T. Ache, A. Sadiq, I. Galadima, K.

Gamaliel (2014). Quality assessment of different brands of dichlofenac tablets in some Pharmacy stores in Abuja. *African Journal of Pharmacy and Pharmacology* 8 (37): 924-928.

21. British Pharmacopoeia, (2005). International Edition, Royal Pharmaceutical Society of Great Britain, CD.

22. United States pharmacopeia (2013). The National formulary: United States Pharmacopeial convention, Inc.; Rockville. pg 2030.

23. United States Pharmacopoeia, (2004). 27th edition, The USP convention Inc, Rockyille, Canada: 2278-2396.

24. W. Chansuda., L.P. Amy, H. Walther, S. Meshnick (2002). Epidemiology of drug resistant malaria. *Lancet Infectious Disease*, 2:209-218.