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Dissolution Profiles of Twelve Brands of Sulphadoxine Pyrimethamine in the Nigerian Market

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ABSTRACT

With the introduction of artemisinin-combination therapies (ACTs) as the standard recommended treatment for falciparum malaria by the World Health Organization (WHO) and its adoption by most malaria-endemic countries like Nigeria, the only available treatment option for pregnant women with malaria became sulphadoxine–pyrimethamine (SP). Several brands of SP have emerged in the local Nigerian and African market. An in vitro dissolution study was conducted on twelve brands of SP in three different dissolution media. The twelve brands of SP were obtained from both the public and private medicine outlets in Nigeria. They were subjected to dissolution in three dissolution media at pH values of 1.2, 4.5, and 6.8. The dissolution samples were assayed for the drug compounds using a double-beam ultraviolet spectrophotometer. The dissolution test results were subjected to analysis of variance (ANOVA) and analyses of difference (f_1) and similarity factors (f_2). Five of the selected twelve brands failed to meet the USP dissolution requirements for immediate-release tablets. Seven of twelve brands had dissimilar dissolution profiles.

INTRODUCTION

Currently, about 2 million deaths per year worldwide are due to plasmodium infections. The majority occur in children under five years of age and pregnant women in sub-Saharan African countries (*1*). Country-specific studies reveal that Nigeria has the largest burden of malaria in Africa (*2*). The emergence of chloroquine resistance necessitates the use of sulphadoxine–pyrimethamine (SP) and artemisinin-combination therapies (ACTs) as first line treatment in most malaria-endemic countries. The SP combination remains the one all-important option for safe malaria treatment in pregnancy. Some countries now recommend that SP be used in combination with chloroquine, amodiaquine, or both (*3*).

Many developing countries like Nigeria do not have an effective means of regulatory control, especially in the aspect of post marketing surveillance activities that involve strict monitoring of the quality, safety, and efficacy of generic drug products in the market. This results in the distribution of substandard and counterfeit drug products to innocent members of the public. A World Health Organization guideline for the registration, marketing authorization, and quality control of generic pharmaceutical products is available to assist countries in organizing and managing their regulatory activities (*4*).

Generic drug products must satisfy the same standards of quality, efficacy, and safety as those applicable to the innovator product. The release of active pharmaceutical ingredients from drug product, the dissolution of the drug under physiological conditions, and the permeability across the gastrointestinal tract determines, to some extent, drug adsorption. In vitro dissolution serves as a lead in assessing in vivo drug product performance for pharmaceutical products containing Biopharmaceutics Classification Scheme (BCS) Class I APIs (high solubility, high permeability) and conditionally those containing BCS Class II (low solubility, high permeability) and Class III (high solubility, low permeability) APIs. Dissolution testing serves as a tool to distinguish between acceptable and unacceptable products (*5*); it is also used to assess the lot-to-lot quality of a drug product and guide the development of new formulations (*6*).

According to a World Health Organization (WHO) report (*7*), sulphadoxine is regarded as a highly soluble API with no sufficient permeability data. It is placed in BCS Class I, while pyrimethamine has a borderline solubility (<0.1 mg/mL) and a low permeability, placing it in BCS Class III. No biowaiver is recommended for the combination of these two APIs; however, it is also recommended that the combination be tested according to pyrimethamine requirements.

The goal of this study was to determine the dissolution profiles of twelve brands of sulphadoxine–pyrimethamine tablets found in the Nigerian market and compare them with that of a reference standard tablet in three different dissolution media, mimicking in vivo conditions as much as possible to obtain baseline data for establishing similarity in profiles among the various brands and the reference standard tablet.

MATERIALS AND METHODS

Twelve different brands of sulphadoxine–pyrimethamine tablets (Table 1) sourced from both private and

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Table 1. Brands of Sulphadoxine–Pyrimethamine Used

Brand No.	Brand Name	Mamufacturing Date	Expiry Date	Batch No.	Country of Origin
1	MALDOX	9/08	9/11	3650M	NIGERIA
2	VITADAR	2/08	1/12	VITK8004	INDIA
3	EVAMAL	6/08	5/11	8002	NIGERIA
4	RIDMAL	5/08	4/11	VM807	INDIA
5	LARIDOX	08/08	7/11	8019	INDIA
6	MALWIN	4/08	3/11	VM–804	INDIA
7	MALAREICH	12/07	11/10	670049	INDIA
8	FRALOMIN	5/07	4/11	SPF701	INDIA
9	GOMAXINE	8/08	7/11	EO2310	INDIA
10	EFRODAR	8/08	7/11	H–027	PAKISTAN
11	FANSIDAR	04/07	04/12	26047	NIGERIA
12	SURAMEX	11/08	10/11	IY1335	NIGERIA

public drug outlets in Nigeria were used. Three tablets of each brand were individually weighed before use in the dissolution studies. The reference standard tablets of sulphadoxine–pyrimethamine were supplied with the minilab, a mobile mini-laboratory for rapid medicine quality verification and counterfeit medicines detection targeted for use in developing countries from Global Pharma Health Fund (GPHF) of Germany. All solvents and reagents used were of analytical grade and freshly prepared.

Dissolution Media

The three dissolution media were 0.1 N hydrochloric acid and phosphate buffers at pH 4.5 and 6.8. The preparations of pH 6.8 phosphate buffer and 0.1 N HCl were from USP XXIII. Phosphate buffer pH 4.5 was prepared by adjusting 20 mM disodium hydrogen phosphate to pH 4.5 with orthophosphoric acid. These media were selected based on the FDA guidance for industry and the need to meet the criteria for biowaiver (*8*).

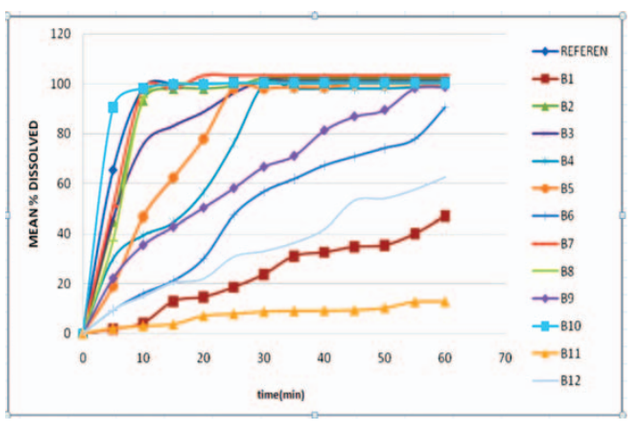

Figure 1. Dissolution profiles of sulphadoxine reference standard and twelve brands in 0.1 N HCl.
REFEREN: Reference Standard
B1: Brand 1, etc.

Dissolution Test Apparatus

Dissolution was carried out using the RC–6 dissolution test Apparatus 2, with six vessels of 1-L capacity made by Tianjin (China) in three replicates of each brand. The dissolution media were maintained at 37 ± 0.5 °C and 75 ± 1 rpm. In all the experiments, 5 mL of dissolution sample was withdrawn every 5 min for 60 min and replaced with an equal volume of medium to maintain sink condition. Samples were filtered and assayed using a Shimadzu UV–1650 PC spectrophotometer. The concentration of each sample was determined from a calibration curve obtained using sulphadoxine–pyrimethamine reference standard.

Assay

Calibration curves were obtained using serial dilutions of a solution of the sulphadoxine–pyrimethamine reference standard in the three dissolution media after scanning the solutions from 190 to 400 nm to obtain the maximum wavelengths of absorption for both active

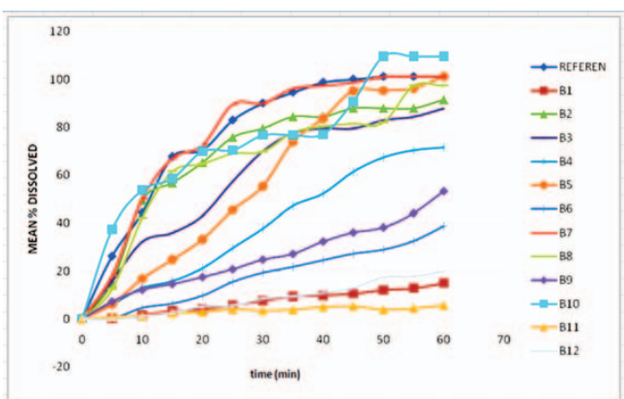

Figure 2. Dissolution profiles of pyrimethamine reference standard and twelve brands in 0.1 N HCl.

Table 2. Dissolution Data in Three Different Media after 30 min

	Sulphadoxine in 0.1 N HCl			Pyrimethamine in 0.1 N HCl			Sulphadoxine in pH 6.8 Phosphate Buffer		
	% Dissolved (mean ± SD)	f_1	f_2	% Dissolved (mean ± SD)	f_1	f_2	% Dissolved (mean ± SD)	f_1	f_2
REFERENCE	100.2 ± 3.4			90.0 ± 3.9			99.80 ± 3.70		
BRAND 1	23.80 ± 3.59	67.50	35.45	7.70 ± 3.54	90.70	29.57	45.10 ± 3.80	36.10	49.06
BRAND 2	102.40 ± 8.65	2.20	96.32	79.40 ± 3.75	14.70	68.45	98.20 ± 2.54	0.00	100.00
BRAND 3	101.10 ± 6.13	0.90	99.29	69.90 ± 4.73	19.70	62.36	23.50 ± 2.82	66.60	35.81
BRAND 4	99.80 ± 4.68	2.00	96.88	37.80 ± 2.07	47.40	43.59	90.60 ± 2.07	1.60	97.90
BRAND 5	98.20 ± 1.87	0.40	98.18	55.30 ± 4.28	35.10	67.47	99.10 ± 2.92	0.70	99.57
BRAND 6	56.8 ± 6.32	32.80	51.00	19.30 ± 2.65	75.70	33.46	67.50 ± 6.00	13.60	69.62
BRAND 7	103.5 ± 4.09	3.30	92.98	90.00 ± 3.22	1.30	98.57	94.4 ± 4.09	0.60	99.68
BRAND 8	100.4 ± 6.50	0.20	99.96	69.80 ± 6.91	18.70	63.47	100.40 ± 6.00	0.60	99.68
BRAND 9	66.7 ± 6.53	18.90	62.79	24.90 ± 4.47	67.70	35.91	90.60 ± 2.00	0.00	100.00
BRAND 10	100.4 ± 6.32	0.20	99.96	76.60 ± 2.64	22.10	59.98	100.40 ± 6.56	0.60	99.68
BRAND 11	8.9 ± 3.75	90.70	29.04	3.60 ± 7.12	95.70	28.40	8.80 ± 3.25	89.70	29.37
BRAND 12	33.0 ± 6.18	58.30	38.62	9.50 ± 5.50	87.60	30.31	76.60 ± 3.22	3.50	92.36

ingredients in the three media. Regression equations for the active ingredients in the different media were used to determine the percentage dissolved per time using absorbances obtained at 215 nm for sulphadoxine and 272 nm for pyrimethamine in pH 6.8 phosphate buffer, 212 nm for sulphadoxine and 272 nm for pyrimethamine in pH 4.5 phosphate buffer, and 210 nm for sulphadoxine and 268 nm for pyrimethamine in 0.1 N HCl.

Data Analysis

The UV–Probe software (Shimadzu) was used to program the spectrophotometer and to acquire and process the primary data. Excel (Microsoft Corp., USA) was used to calculate the percent dissolved of the APIs for three individual tablets, the mean and standard deviation, and the significance of Analysis of Variance (ANOVA). The similarity factors (f_2) and difference factors (f_1) were computed.

RESULTS AND DISCUSSION

The dissolution data obtained for the twelve brands of sulphadoxine–pyrimethamine tablets in the three dissolution media after 30 min and their calculated f_1 and f_2 values are shown in Table 2. There is high variation in the dissolution of the APIs in these brands in the chosen dissolution media.

The USP specifies for both APIs that the amount of drug released in a dissolution experiment after 30 min should not be less than 60% of the labeled amount in pH 6.8 phosphate buffer. Brands 2, 4, 5, 7, 8, 9, and 10 complied with this requirement for both active ingredients analyzed, while Brand 1 had a total release below 50% and Brand 11 below 20% after 60 min, as shown in Figures 1–6.

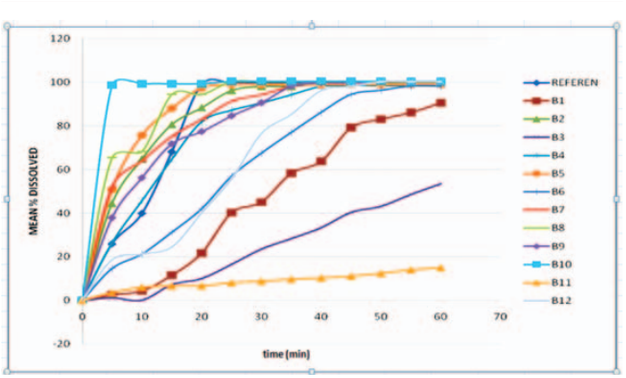

Figure 3. Dissolution profiles of sulphadoxine reference standard and twelve brands in pH 6.8 phosphate buffer.

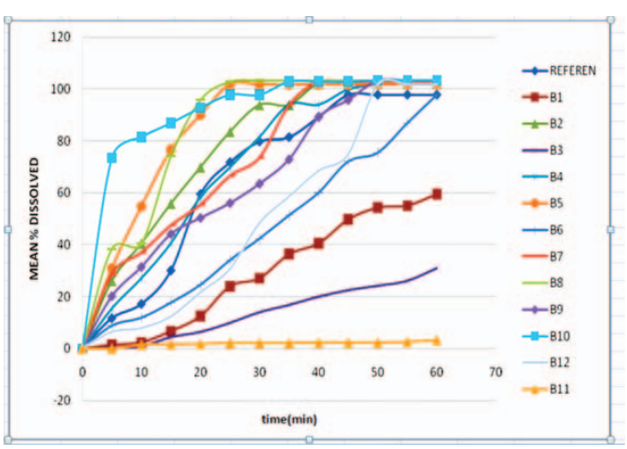

Figure 4. Dissolution profiles of pyrimethamine reference standard and twelve brands in pH 6.8 phosphate buffer.

Table 2. Dissolution Data in Three Different Media after 30 min (continued)

	Pyrimethamine in pH 6.8 Phosphate Buffer			Sulphadoxine in pH 4.5 Phosphate Buffer			Pyrimethamine in pH 4.5 Phosphate Buffer		
	% Dissolved (mean ± SD)	f_1	f_2	% Dissolved (mean ± SD)	f_1	f_2	% Dissolved (mean ± SD)	f_1	f_2
REFERENCE	79.80 ± 3.90			78.80 ± 2.90			77.80 ± 3.80		
BRAND 1	27.10 ± 4.86	54.70	42.50	26.10 ± 4.76	54.60	42.73	27.50 ± 3.86	52.60	43.13
BRAND 2	93.90 ± 3.76	14.60	70.50	90.90 ± 2.76	13.80	71.82	92.90 ± 4.76	14.00	71.17
BRAND 3	14.00 ± 5.55	77.60	34.92	12.00 ± 4.52	76.00	35.55	15.00 ± 5.45	76.90	34.92
BRAND 4	81.50 ± 4.71	5.20	88.96	80.50 ± 4.00	6.10	86.62	81.90 ± 4.61	6.70	84.95
BRAND 5	101.70 ± 2.00	13.90	71.50	100.50 ± 1.56	13.80	71.82	101.50 ± 1.57	12.90	72.83
BRAND 6	42.40 ± 4.33	32.70	53.56	43.40 ± 4.23	31.80	54.34	43.30 ± 3.55	30.90	55.93
BRAND 7	73.70 ± 1.40	15.20	69.62	73.90 ± 1.40	15.40	69.62	73.50 ± 1.70	12.70	73.18
BRAND 8	103.40 ± 2.34	15.80	68.17	102.60 ± 4.34	16.20	68.59	103.60 ± 2.94	15.20	69.47
BRAND 9	63.6 ± 3.75	0.00	100.00	63.70 ± 3.05	1.60	98.36	63.70 ± 3.75	0.70	99.68
BRAND 10	97.80 ± 1.57	15.20	69.62	97.90 ± 4.57	15.10	69.92	97.80 ± 3.57	12.90	72.80
BRAND 11	2.20 ± 3.00	97.40	29.99	2.15 ± 2.00	97.20	30.24	2.10 ± 7.00	97.20	29.84
BRAND 12	48.40 ± 4.62	23.30	60.78	48.90 ± 3.62	23.50	60.78	48.45 ± 3.62	17.80	59.79

According to the FDA Guidance for Industry *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System* (*8*), for Class I and in some cases Class III drugs, 85% dissolution in 15 min ensures that the bioavailability of the drug is not limited by dissolution. Brands 2, 5, 7, 8, and 10 met this condition with respect to sulphadoxine release in the three media used, and Brand 7 released over 85% of both active ingredients in 15 min.

The percentages of both active ingredients dissolved in all twelve brands were statistically compared with that of the reference standard using one-way ANOVA. The analyses were conducted for all time points. The results of ANOVA (Table 3) reveal that the profiles of brands 2, 5, 7, 8, and 10 show no significant difference from that of the reference standard at 95% confidence level; while those of the remaining seven brands differ significantly.

With the understanding that no single comparison approach is widely accepted to determine similarity of dissolution profiles (*9*), the dissolution profiles of the different brands were further compared using a model-independent approach. Difference factors (f_1) and similarity factors (f_2) were employed using the following equations:

$$f_1 = \left\{\left[\sum_{t=1}^{n}|R_t - T_t|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} \times 100$$

$$f_2 = 50 \cdot \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

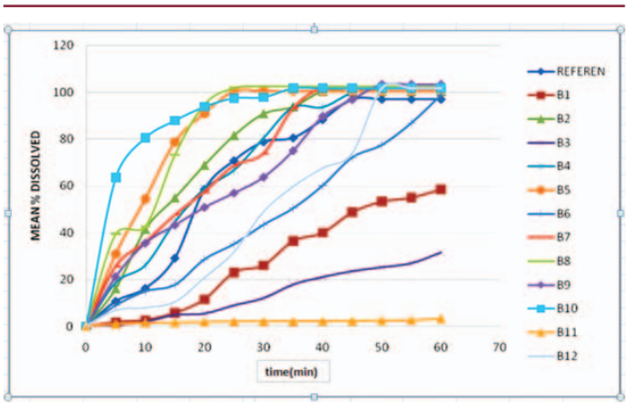

Figure 5. Dissolution profiles of sulphadoxine reference standard and twelve brands in pH 4.5 phosphate buffer.

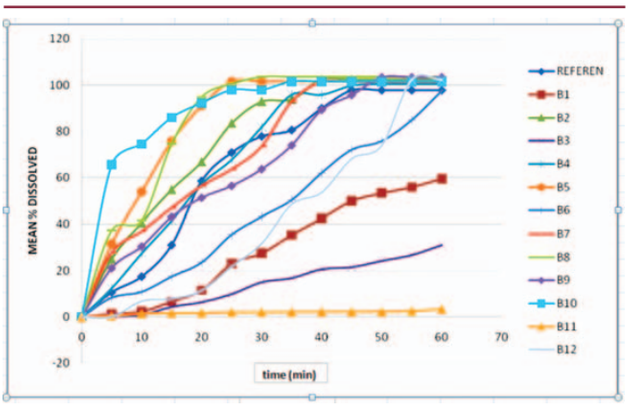

Figure 6. Dissolution profiles of pyrimethamine reference standard and twelve brands in pH 4.5 phosphate buffer.

Table 3. ANOVA of the Profile of the Different Brands by the Profile of the Reference at 0.05 or 95% Confidence Interval

BRAND	Sulphadoxine in 0.1 N HCl		Pyrimethamine in 0.1 N HCl		Sulphadoxine in pH 6.8 Phosphate Buffer		Pyrimethamine in pH 6.8 Phosphate Buffer		Sulphadoxine in pH 4.5 Phosphate Buffer		Pyrimethamine in pH 4.5 Phosphate Buffer	
	F	SIG	*F*	SIG	*F*	SIG	*F*	SIG	*F*	SIG	*F*	SIG
1	4.506	0.044	12.474	0.076	13.175	0.003	39.147	0.006	37.358	0.006	38.882	0.006
2	409.681	0.000	443.216	0.007	490.079	0.000	478.4	0.000	1090.834	0.000	1031.778	0.000
3	32.367	0.000	114.382	0.009	490.079	0.000	10.512	0.039	12.898	0.630	8.685	0.051
4	5.407	0.029	162.471	0.006	36.483	0.000	1499.414	0.000	1427.256	0.000	8695.523	0.000
5	19.812	0.001	149.934	0.007	482.360	0.000	748.745	0.000	715.681	0.000	747.181	0.000
6	3.979	0.058	7.044	0.130	13.548	0.003	8.800	0.050	8.575	0.052	9.409	0.046
7	422.475	0.000	611.645	0.000	556.01	0.000	1127.5	0.000	1123.495	0.000	1089.596	0.000
8	28171.047	0.000	8.391	0.111	362.224	0.000	851.154	0.000	794.787	0.000	846.956	0.000
9	4.547	0.043	3.882	0.222	29.185	0.000	85.337	0.002	11005	0.001	80.768	0.002
10	675.812	0.000	654.947	0.000	791.3	0.000	2244.598	0.000	791.505	0.000	303.174	0.000
11	5.142	0.032	3.151	0.264	15.826	0.00	3.449	0.168	2.063	0.298	1.995	0.308
12	3.133	0.093	11.811	0.080	11.553	0.005	10.219	0.041	9.577	0.045	9.112	0.048

F: calculated *F*-ratio
SIG: level of significance

where n is the number of time points, R_t is the dissolution value of reference product at time t, and T_t is the dissolution value for the test product at time t.

For two dissolution profiles to be considered similar, f_1 should be between 0 and 15 and f_2 should be between 50 and 100 (*10*). The f_1 and f_2 values obtained for all brands in the three media further support the similarity of the dissolution profiles of brands 2, 5, 7, 8, and 10 to that of the reference product. It may be appropriate that drug regulatory authorities in developing countries like Nigeria adopt in vitro dissolution studies to serve as a guide to which brand(s) of a particular class of medicine to license, thereby reducing the proliferation of substandard brands.

CONCLUSION

The findings of this study show comparatively high variation in the release profiles of the different brands of sulphadoxine–pyrimethamine tablets found in the Nigerian market even though preliminary chemical tests carried out prior to dissolution revealed that all the brands used actually contained the label amount of active ingredients within official limits. The failure of 41.7% of the selected brands to meet the required standards suggests the need for a more effective quality control–quality assurance system at the level of the National Regulatory Body before drug products are granted marketing authorization, more so as all the brands used in this study had been licensed.

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