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Comparative qualitative and quantitative assessment of selected brands of Co-trimoxazole tablets and suspensions procured from Lagos State, Nigeria using first derivative UV spectroscopy and HPLC

Oluwakemi Charles-Okhe, Oluwadamilola M Kolawole * and Boladale O Silva

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.

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Abstract

Background: The use of High-Performance Liquid Chromatography (HPLC) to evaluate the quality of co-trimoxazole formulations is relatively expensive, and First Derivative UV Spectroscopic (FDS) technique may be alternatively used. There are no studies evaluating the quality of locally manufactured sulphamethoxazole/trimethoprim (SMX/TMP) dosage forms procured from Lagos, Nigeria. The aim of the work was to evaluate the quality attributes of selected co-trimoxazole tablets and suspensions marketed in Lagos, Nigeria, and determine their API content using first derivative UV spectroscopy and HPLC.

Methods: Ten samples each of Co-trimoxazole tablets and suspensions were procured and their quality attributes were evaluated using standard pharmacopeial methods while their drug content was quantified using FDS and HPLC methods.

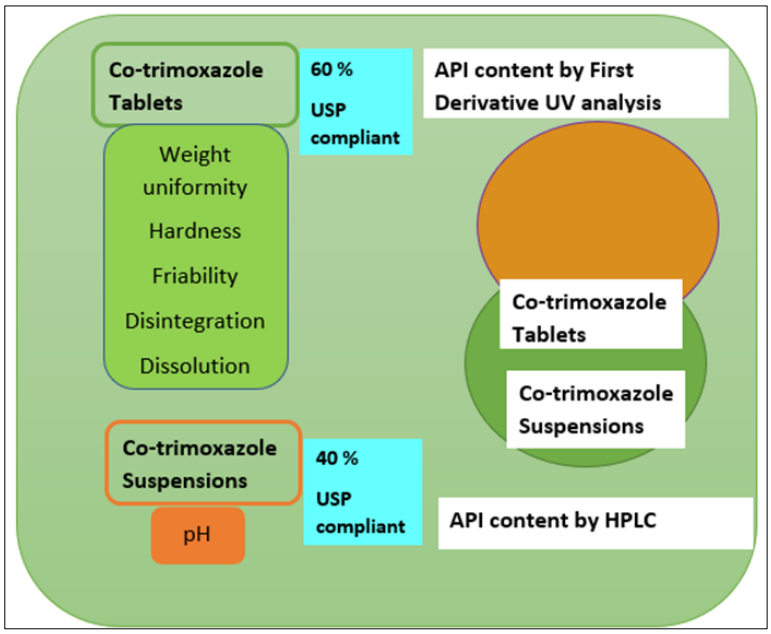
Results: 70 % of the tested tablet as well as all the suspension samples exhibited satisfactory quality attributes. In terms of the drug content assay, 60 % of the studied tablets and 40 % of the suspension brands were USP-compliant. The SMX and TMP content of the suspension formulations using FDS technique was generally less than that obtained using the HPLC method, suggesting that the active ingredients might have degraded. Conversely, there was good correlation in the drug content of the tablets using FDS and HPLC.

Conclusion: First Derivative UV spectroscopy may be suitable to quantify the drug content of co-trimoxazole tablets while HPLC technique may be preferable for co-trimoxazole suspensions. Furthermore, stringent regulatory monitoring of the marketed co-trimoxazole products is required to assure the quality, safety and efficacy of marketed co-trimoxazole products, thereby safeguarding public health.

Keywords: Sulphamethoxazole; Trimethoprim; First Derivative UV Spectrophotometry; HPLC; Qualitative and Quantitative Assessment

* Corresponding author: Oluwadamilola M. Kolawole
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.

Graphical abstract



1. Introduction

Pharmaceutical products intended to treat, prevent, diagnose a disease or improve public health, must be safe, efficacious and of good quality, and the United Nations Sustainable Development Goals (Goal No. 3.8) advocates for improved access to these medicines in order to promote overall health and well-being of the populace [1 ,2]. The quality of antibiotic medicines are regarded as poor if they are substandard, counterfeit or degraded [3], and the integrity of these drug products may be compromised due to poor manufacturing processes as well as inappropriate transport or storage conditions [4]. The oral or systemic administration of these substandard or adulterated antibiotics result in therapeutic failure, prolonged illness, increased healthcare expenses, adverse events, and death [5].

The multi-drug resistance reported with the use of anti-infective agents has been associated partly with the lack of chemical equivalence between various marketed brands. The Southeast Asia and Africa appeared to be the most affected by counterfeit antibiotics, with WHO Survey revealing that 28 % of the studied antibacterial agents did not comply with pharmacopeial quality standards [6].

P-amino benzoic acid (PABA) is a substrate which generates folic acid, protein and nucleic acid within bacterial cells. Sulphamethoxazole (SMX) is an antibacterial sulphonamide which is structurally similar to PABA. SMX competes with PABA, and inhibits dihydrofolic acid synthesis within the bacterial cells [7]. In addition, Trimethoprim (TMP), a second antibacterial agent, binds selectively to bacterial dihydrofolate reductase and prevents the reduction of dihydrofolic acid to tetrahydrofolic acid, without interfering with mammalian enzymes [8].

A fixed dose combination of sulphamethoxazole and trimethoprim was introduced in the 20th century to treat a broad spectrum of bacterial infections, including urinary tract infections, gram-negative sepsis, respiratory infections, enteric infections, sexually transmitted infections, and opportunistic infections common with HIV positive individuals [9-10]. The drug formulation could promote synergism between the two antibiotics, thereby improving the therapeutic outcomes of patients with bacterial infections [11].

The estimation of sulphamethoxazole and trimethoprim content of co-trimoxazole tablets and suspensions using ordinary, zero-order UV spectrophotometry is difficult because the two components give overlapping spectral bands [12]. The derivative UV spectroscopy is a simple, rapid and modern analytical technique that allows both qualitative and quantitative information to be generated from the spectra of drug mixture composed of overlapped absorption bands [13]. This technique permits the quantification of one analyte in the presence of a second chemical agent without prior separation of components. The amount of each component of the mixture is quantified based on the resolution of their absorption signals efficiently as well as avoidance of interferences from the spectral background [13].

The use of HPLC to analyze co-trimoxazole tablets and suspensions containing sulphamethoxazole and trimethoprim was recommended by the BP and USP because it could detect both drugs at a particular wavelength but the analytical technique is quite expensive and thus it is not routinely used for the estimation of the API content of co-trimoxazole dosage forms [12]. Nevertheless, Alzomor et al. used HPLC technique for the qualitative and quantitative assessment of co-trimoxazole tablets procured from Yemeni market, with trimethoprim and sulphamethoxazole detected at wavelength of 254 nm [8]. However, the quantification of the drug content of cotrimoxazole suspension was not within the scope of their research.

Nwakile and co-workers were not detailed about the solvent as well as method used to prepare co-trimoxazole suspensions procured from the South Eastern part of Nigeria, prior to UV analysis. Also, they did not state the wavelength of maximum absorption of the constituent sulphamethoxazole and trimethoprim in the tested drug product [1]. In a latter study, Frimpong and coworkers used UV-Vis spectroscopy to evaluate the SMX and TMP content of cotrimoxazole suspension marketed in Ashanti area of Ghana, with TMP and SMX maximally absorbed at wavelengths of 204 nm and 257 nm, respectively [11]. Recently, Irungu et al. studied the quality attributes of selected co-trimoxazole suspension brands marketed in Nairobi, Kenya using HPLC, with SMX and TMP detected at a wavelength of 254 nm [3].

Over the last decade, researchers have consistently evaluated the quality attributes and drug content of marketed antibiotic products because these class of essential medicines frequently contains suboptimal levels of the active pharmaceutical ingredients due to the activities of dubious manufacturers that adulterate the drug products. For example, Nwakile et al., (2011) reported that 23.3 % out of the 9 brands of co-trimoxazole suspensions procured from selected states in the South-eastern part of Nigeria failed microbial load test, though all the samples exhibited satisfactory physicochemical attributes [1]. In Papua Guinea, 90 % of 360 antibiotic tablets and capsules analysed for API content, did not comply with pharmacopeial reference ranges [14]. Khuluza reported that five (45 %) out of eleven co-trimoxazole tablets sampled from Malawi were not BP-compliant based on their API content [15]. Fadeyi and co-workers reported that 60 % of 15 co-trimoxazole tablet samples procured from Ghana, Nigeria and United Kingdom were not USP-compliant in terms of drug content [16]. Lawal et al. (2019) reported that 59 % of 17 co-trimoxazole tablet brands marketed in Katsina, Nigeria passed the API content test based on HPLC analysis [17]. Recently, Irungu et al. (2021) revealed an improvement in the quality of co-trimoxazole suspension brands marketed in Nairobi, Kenya, with 86.8 % of the 106 co-trimoxazole suspension samples complying with pharmacopeial specification for API content while 70.6 % of them exhibited satisfactory pH values [3].

To the best of our knowledge, there are no studies where both UV and HPLC techniques have been used comparatively to evaluate the API content of co-trimoxazole tablets and suspensions. Moreover, the quality attributes of co-trimoxazole tablets and suspensions procured from Lagos, Nigeria have not been evaluated. Furthermore, the possibility of using cost-effective First Derivative UV spectroscopy as an alternative technique to HPLC, to quantify the API content of co-trimoxazole formulations may be valuable in resource-limited and developing countries.

The main aim of this study was to evaluate the quality attributes of selected co-trimoxazole tablet and suspension brands procured from reputable community pharmacies in Lagos, Nigeria. In addition, their API content will be evaluated using First Derivative UV spectroscopy and HPLC.

2. Material and methods

2.1. Materials

Sulphamethoxazole (99.9 %) and Trimethoprim (99.5 %) reference powder was obtained from Gemini Pharmaceuticals Ltd, Lagos, Nigeria. HPLC grade acetonitrile, methanol, triethylamine, glacial acetic acid, absolute ethanol, sodium acetate and filter papers (Whatman No. 1) were procured from Sigma Aldrich, Germany. All chemicals and solvents were of analytical grade.

2.2. Sample Collection

Co-trimoxazole tablets and suspension brands (10 samples each) that have been registered with food and drug regulatory authority in Nigeria (NAFDAC) were procured from different retail pharmacies in Lagos, Nigeria and the samples were coded so that the identity of the manufacturer remains anonymous. Their quality assessment and API quantification were carried out at least six months prior to the expiration of the drug products.

The sample code, dosage form, and NAFDAC Registration numbers of the sampled co-trimoxazole tablets and suspensions are presented below (Table 1).

Table 1 Characteristics of the investigated co-trimoxazole tablet and suspension samples

Sample Code	Dosage form of samples	NAFDAC Reg. No.
A_1	Tablet	04-1959
A_2	Tablet	04-1959
B_1	Tablet	04-2117
B_2	Tablet	04-2117
C_1	Tablet	04-0267
C_2	Tablet	04-0267
D_1	Tablet	04-5567
D_2	Tablet	04-5567
E_1	Tablet	04-5567
E_2	Tablet	04-5567
F_1	Suspension	04-1810
F_2	Suspension	04-1810
G_1	Suspension	04-2377
G_2	Suspension	04-2377
H_1	Suspension	04-1456
H_2	Suspension	04-1456
I_1	Suspension	04-4135
I_2	Suspension	04-4135
J_1	Suspension	04-0152
J_2	Suspension	04-0152

N.B: All tablets and suspensions contained SMX 400 mg /TMP 80 mg and SMX 200 mg /TMP 40 mg per 5 mL suspension, respectively.

2.3. Methods

2.3.1. Quality Evaluation of Co-trimoxazole tablets and suspensions

The quality attributes of co-trimoxazole tablets such as uniformity of weight, hardness, friability, disintegration, and dissolution profile were evaluated while the pH of the co-trimoxazole suspensions was determined according to the United States pharmacopoeial (USP) standards and protocols [18]. The SMX and TMP contents of co-trimoxazole tablets and suspensions were evaluated using First Derivative UV Spectrophotometry and HPLC analysis.

Uniformity of weight test

In order to evaluate the uniformity of weight of the tablets, 20 tablets were randomly selected from the various brands and individually weighed (W1). The recorded weights were compared with the average weight of all studied tablets (WA).

$$\% \text{ weight variation} = \text{WA} - \text{W1} \ /\text{WA} * 100$$

The sample complies with USP standard if the weight of no more than two tablets out of the 20 tested tablets deviate from the average weight by 5 % and if none of the tested tablets differ from the average weight of tablets by more than 10 %.

Hardness test

Four tablets were randomly chosen from each tablet brand and the hardness of the tablets was evaluated using the hardness tester. The average of four hardness values was noted and its standard deviation calculated.

Friability test

Ten tablets from each brand were initially weighed on an analytical balance and placed in the drum of the friability tester and rotated at 25 rpm for 4 min. Then, the tablets were removed and re-weighed after 100 rotations. The percent friability was evaluated as a function of the percentage weight loss of the tablets.

$$\% \text{ Friability} = (W1 - W2)\ /W1 * 100$$

Where W1 = Initial weight of the 10 tablets; W2 = Final weight of the 10 tablets after testing.

The batch of tablets complied with pharmacopeial standard if the friability of the tablets is less than 1 %.

Disintegration test

The disintegration profile of the tablets was evaluated using USP method [18]; six tablets were maintained in purified water (700 mL) at 37 ^{o}C for 15 min. The disintegration time was indicated as the time when tablet disintegration occurred. The batch of product passed if ≤ 2 tablets failed the test after evaluating a maximum of 18 tablets. Additional tablets (12) do not need to be evaluated if the first six tablets passed the test.

Dissolution test

The dissolution pattern of the tablets was studied by placing six tablets from each brand of tablet in 900 mL of 0.1 M HCl, contained in the USP II paddle apparatus operated at 75 rpm for 60 min. For the batch of products to pass the test, at least 70 % of SMX and TMP contained in the tablets must be detected in the dissolution medium after the study period.

2.3.2. Drug Quantification

Standard SMX/TMP solutions in 0.1 M HCl were prepared such that SMX (3 to 5 µg/mL) and TMP (0.6 to 1.0 µg/mL) were present in each sample at a ratio of 5:1, and the solutions were analysed using HPLC to generate a calibration curve, with a correlation coefficient of ≥ 0.9. This graph was used to quantify the amount of drug dissolved in the dissolution medium during the study period.

2.3.3. pH for co-trimoxazole suspension samples

The pH of each reconstituted suspension samples (200 mg/5 mL) was determined with a calibrated Thermo Orion Star A211 pH meter (Thermo Fisher Scientific, UK). The pH of the tested samples will pass pH test if it complies with standards specified by the USP, for oral drug suspensions (5.0 to 6.5) [11].

2.3.4. Preparation of SMX and TMP standard stock solutions

The SMX and TMP stock solutions (100 µg/mL each) were prepared separately in aqueous ethanolic solution (10 %), samples refridgerated and used within 10 days of preparation.

2.3.5. Preparation of SMX and TMP standard working solutions

Sodium acetate-acetic acid buffer system was prepared by dissolving sodium acetate (6.8 g) and acetic acid (37 %, 3 mL) in 800 mL distilled water, shaken, pH of the sample adjusted and made up with enough water to produce 1 L buffer solution (pH 4.5). Then, the standard working solutions were prepared by mixing a predetermined volume of the SMX and TMP standard stock solutions, acetate buffer solution, and 90 % ethanol (Table 2). The mixtures were transferred into a volumetric flask and made up to 20 mL mark using water. The resultant solutions contained 15-35 µg/mL of SMX and 3-7 µg/mL of TMP.

In order to evaluate the wavelength of maximum absorption of SMX and TMP, the standard working solutions containing SMX and/or TMP were evaluated using first derivative UV-Vis Spectrophotometer, with samples scanned from 200 nm to 380 nm. The wavelength of maximum absorption (λ max) for SMX corresponds to the zero-crossing point of TMP and vice-versa.

Table 2 Constituents of SMX and TMP working solutions (20 mL) containing 15-35 µg/mL of SMX and 3-7 µg/mL of TMP used for UV-Vis spectroscopic analysis

SMX stock (mL)	TMP stock (mL)	Acetate buffer, pH 4.5 (mL)	90 % ethanol (mL)	Water (mL)	Conc. of SMX (µg/mL)	Conc. of TMP (µg/mL)
3.00	0.60	4.00	2.20	10.20	15.00	3.00
4.00	0.80	4.00	2.20	9.00	20.00	4.00
4.00	1.00	4.00	2.20	7.80	25.00	5.00
5.00	1.20	4.00	2.20	6.60	30.00	6.00
7.00	1.40	4.00	2.20	5.40	35.00	7.00

N.B.: SMX and TMP working solutions intended for HPLC analysis was prepared using triethyl ammonium acetate buffer and methanol instead of sodium acetate-acetic acid buffer system and ethanol used for FDS analysis

The calibration curves for SMX and TMP quantification were generated by plotting the absorbance values of five samples containing 15-35 µg/mL of SMX and 3-7 µg/mL of TMP against their concentration values at the identified wavelengths of maximum absorbance for both drugs; and a mixture of sodium acetate-acetic acid buffer pH 4.5 and aqueous ethanol (90 %) served as the blank solution.

2.3.6. Drug content estimation of co-trimoxazole tablets and suspensions – First Derivative UV spectroscopic method

The amount of active ingredient detected in the co-trimoxazole formulations should be between 90 % to 110 % of the amount of API stated on the product pack. The tablet and suspension samples were prepared and analysed using UV Spectrophotometer operated using the first derivative mode.

2.3.7. Preparation of co-trimoxazole tablet test solution

The tablet stock solution containing 1 mg/mL of SMX and 0.2 mg/mL of TMP was prepared by weighing powdered tablet equivalent to SMX (100 mg) and TMP (20 mg), and transferring it into 100 mL volumetric flask. Ethanol (90 %; 35 mL) was added to the SMX/ TMP powdered mixture, and sonicated for 15 min on an ultrasonic bath. Then, the drug solution was made up to 100 mL with ethanol, filtered and labelled. The tablet stock solution was mixed with acetate buffer and ethanol, and the mixture was made up to 20 mL solution in a volumetric flask with water, resulting in a final SMX and TMP concentration of 25 µg/mL and 5 µg/mL, respectively in the tablet solution. Then, Agilent 8453 UV-visible single beam spectrophotometer was used to obtain the spectra and absorbance values of various tablet test solutions at the wavelength of maximum absorption (λ max) for SMX and TMP.

2.3.8. Preparation of co-trimoxazole suspension test solution

Co-trimoxazole suspension typically contains 200 mg of SMX and 40 mg of TMP per 5 mL of the reconstituted suspension. The suspension powder was reconstituted with distilled water and shaken for homogeneity. The suspension (2.5 mL), which contained 100 mg SMX and 20 mg TMP, was secured in a 100 mL volumetric flask and mixed with 90 % ethanol (35 mL). The mixture was sonicated for 15 min on an ultrasonic bath and made up to 100 mL volume with ethanol (90 %) and filtered to generate the suspension stock solution containing 1 mg/mL SMX and 0.2 mg/mL of TMP. Then, the suspension stock solution (0.5 mL) was mixed with the acetate buffer solution (4 mL) and 90 % ethanol (2.2 mL), and made up to volume with water in 20 mL volumetric flask, with resultant test solution containing 25 µg/mL of SMX and 5µg/mL of TMP. Similar UV machine was used to measure the absorbance of the suspension samples at the wavelength of maximum absorption for sulphamethoxazole and trimethoprim.

2.3.9. Drug content of co-trimoxazole tablets and suspensions- HPLC method

A calibration curve was generated by analyzing various concentrations of SMX (15-35 µg/mL) and TMP (3-7 µg/mL) prepared using methanol and triethyl ammonium acetate buffer (TrEAA). The triethyl ammonium acetate buffer system was prepared by dissolving 6 mL of glacial acetic acid and 3 mL of Triethyl amine and made up to 1 L solution using water. The tablets and suspensions samples containing SMX (25 µg/mL) and TMP (5 µg/mL) were prepared as previously described for FDS analysis but methanol and triethyl ammonium acetate buffer system was used for sample preparation.

The resultant drug solutions were analyzed using HPLC instrument (Agilent 1200) coupled with the quaternary pump and UV detector, operated at a wavelength of 254 nm. The drug content of the tablets and suspensions was analysed by

injecting aliquot volume of the test solutions (10 µL) into the C18 reverse phase column, 150 mm x 4.6 mm x 5 µm column (Zorbax XDB, Sigma Aldrich, UK) maintained at 25 ^{o}C. The mobile phase consists of triethyl ammonium acetate buffer, methanol and acetonitrile (70:20:10), run at a constant flow rate of 1 mL/min, for 7 min.

2.4. Statistical analysis

All the experiments were carried out in triplicates. One-way ANOVA statistical method was used to evaluate if there were any significant statistical differences between the API content of the co-trimoxazole tablets and suspensions analysed using the First Derivative UV spectroscopic and HPLC method of analysis, and $p < 0.5$ depicts significant statistical differences between datasets.

3. Results and discussion

The tablet and suspension brands used for this study comprise of locally manufactured brands that were within their shelf-life as at the time of study. Various compendial and non-compendial quality control tests such as weight uniformity, hardness, friability, disintegration and dissolution tests were carried out on the tablet samples while the pH of the co-trimoxazole suspension samples were assessed in order to evaluate their physicochemical equivalence. In addition, the possibility of using the First derivative UV method as an alternative technique to USP recommended HPLC for the drug content assay of co-trimoxazole tablets and suspensions, was investigated.

Solid dosage forms obtained from different pharmaceutical manufacturers typically exhibit varied quality attributes due to their formulation conditions such as mixing technique, granulation methods as well as the amount and type of excipients incorporated into the formulations [19]. Nevertheless, there is a strong need to ensure that the differences in physicochemical properties such as hardness, friability, disintegration, and dissolution profile are minimized for optimal product performance. Moreover, the finished drug products distributed to the market should comply with pharmacopeial specifications in order to ensure therapeutic efficacy, prevent undesirable toxic effects associated with drug overdose and safeguard public health.

The quality attributes of co-trimoxazole tablet and suspension samples are presented in Table 3.

The pH values of orally administered pharmaceutical formulations dictates their API stability, microbial quality, and physiological suitability [11, 19]. The United States Pharmacopeia recommends that drug suspensions should exhibit pH values between 5.0 and 6.5, and all the studied suspension brands displayed acceptable pH of 5.6 to 6.1. This finding is in good agreement with findings reported by Nwakile et al. (2011)[1] and Frimpong and co-workers [11] where all their studied co-trimoxazole suspension brands passed the pH test.

It is desirable that batches of pharmaceutical tablets exhibit uniform tablet weight because tablet weight variations may be an indication of the non-uniformity in the amount of active ingredient and excipients present in the formulation, which could result in therapeutic failure or toxic effects. The tablets had average weights between 500.2 mg and 576.8 mg, and they exhibited acceptable tablet weight variation of 1.0 ± 0.1 %. The individual weights of two of the studied 20 tablets did not deviate from the average weight by more than 5 % and the differences between the individual tablet weight and the average tablet weights for all the studied tablets was not greater than 10 % [21].

Hardness test evaluates the ability of tablets to withstand stress during handling, packaging and transportation throughout its shelf life [22], and the hardness limit set by USP is 4 to 10 Kgf. The tablets exhibited somewhat satisfactory hardness between 4.9 and 10.9 Kgf. The tablet sample A2 that exhibited hardness of 10.9 Kgf may still be acceptable for human use as it exhibited a disintegration time of 4.9 min, suggesting that the hardness of tablet A2 did not compromise its disintegration. Surprisingly, co-trimoxazole tablets evaluated by Alzomor and co-workers exhibited relatively high hardness values of 9.0 to 23.6 Kgf but they still exhibited disintegration time of 1.0 to 7.5 min [8]. This finding revealed that hardness values may be dependent on the type of hardness tester used for hardness evaluation and the disintegration profile of tablets may be a more decisive method of evaluating their dissolution tendency.

The friability test is a reliable technique to study tablet behavior during packaging, transportation and handling [18, 21]. The tablet samples exhibited satisfactory friability of 0.1 – 0.4 %, as these range of values complies with USP specification (≤ 1 %) [21]. This finding suggests that the tablets would be resistant to chipping during manufacturing processes and patient handling. There was a good correlation between the hardness and friability profile of the studied tablets. Conversely, the hardness and friability profile of co-trimoxazole tablets procured from Yemeni Market in Western Asia did not correlate well as one of the samples with hardness of 10.4 Kgf exhibited unsatisfactory friability of 1.3 % [8].

Table 3 Quality control tests on various brands of Co-trimoxazole tablets and suspensions

Samples	pH± S.D	Average Weight (mg) ± S.D	Weight Variation (%) ± S.D	Hardness (Kgf) ± S.D	Friability (%)) ± S.D	Disintegration time (min) ± S.D
A1	N/A	502.4 ± 7.9	1.0±0.2	4.9±0.1	0.4±0.1	0.6±0.1
A2	N/A	513.7 ± 11.9	1.0 ±0.1	10.9±0.4	0.4±0.1	4.9±0.2
B1	N/A	573.7 ± 8.7	0.9±0.1	6.2±0.2	0.5±0.2	1.0±0.1
B2	N/A	576.9 ± 6.7	0.9±0.1	7.0±0.2	0.5±0.1	1.5±0.1
C1	N/A	522.2 ± 10.6	1.0±0.1	4.4±0.1	0.4±0.1	0.4±0.1
C2	N/A	524.7 ± 6.9	1.0±0.2	7.0±0.2	0.4±0.1	0.3±0.1
D1	N/A	556.8 ± 7.5	0.9±0.2	5.9±0.2	0.3±0.1	4.0±0.2
D2	N/A	576.8 ± 5.4	1.0±0.1	4.9±0.2	0.3±0.1	6.9±0.2
E1	N/A	503.7 ± 4.5	1.0±0.2	9.5±0.3	0.1±0.1	28.2±0.3
E2	N/A	500.2 ± 6.7	1.1±0.2	9.0±0.3	0.1±0.1	1.1±0.1
F1	5.9±0.1	N/A	N/A	N/A	N/A	N/A
F2	5.9±0.2	N/A	N/A	N/A	N/A	N/A
G1	6.0±0.2	N/A	N/A	N/A	N/A	N/A
G2	6.0±0.1	N/A	N/A	N/A	N/A	N/A
H1	5.6±0.2	N/A	N/A	N/A	N/A	N/A
H2	5.7±0.1	N/A	N/A	N/A	N/A	N/A
I1	5.7±0.1	N/A	N/A	N/A	N/A	N/A
I2	6.0±0.1	N/A	N/A	N/A	N/A	N/A
J1	6.1±0.1	N/A	N/A	N/A	N/A	N/A
J2	5.8±0.1	N/A	N/A	N/A	N/A	N/A
Limit	5.0 to 6.5		≤ 5 %	4-10 Kgf	≤ 1 %	< 15 min

A1-E2 are tablets while F1-J2 are suspension dosage forms; N/A = not applicable

Disintegration profile of tablets could be directly related to dissolution and subsequent bioavailability of a drug at target sites within the body because active pharmaceutical ingredients would not be readily available for dissolution if the immediate release tablet does not disintegrate in vivo. The USP specifies that uncoated tablets should completely disintegrate within 15 min. All the tablet brands passed the disintegration test as they exhibited disintegration time of 0.3 – 6.9 min except sample E1 that disintegrated within 28.2 min, despite exhibiting a hardness of 9.5 Kgf. This finding may be due to insufficient amount of disintegrant used to formulate the co-trimoxazole tablets, delaying its disintegration. Surprisingly, one of the co-trimoxazole tablet samples evaluated by Alzomor and co-workers disintegrated within 1 min, despite exhibiting hardness value of 23.6 Kgf. The presence of super-disintegrant in the tablet formulation might have facilitate the disintegration and release of API into the simulant biological fluid.

Dissolution profiles are used to predict the bioavailability of the drug product. The USP specifies that the amount of drug released into the dissolution medium within 60 min should be at least 70 % of the amount of API stated on the product pack. The dissolution study revealed that satisfactory amount of SMX and TMP (72 % - 100 %) were released from 80 % of the studied co-trimoxazole tablet brands within 60 min. On the other hand, all the co-trimoxazole tablets evaluated by Alzomor and co-workers exhibited acceptable dissolution profile as 76.5-101.9 % of SMX and 98.5-103.7 % of TMP was released within 60 min [8].

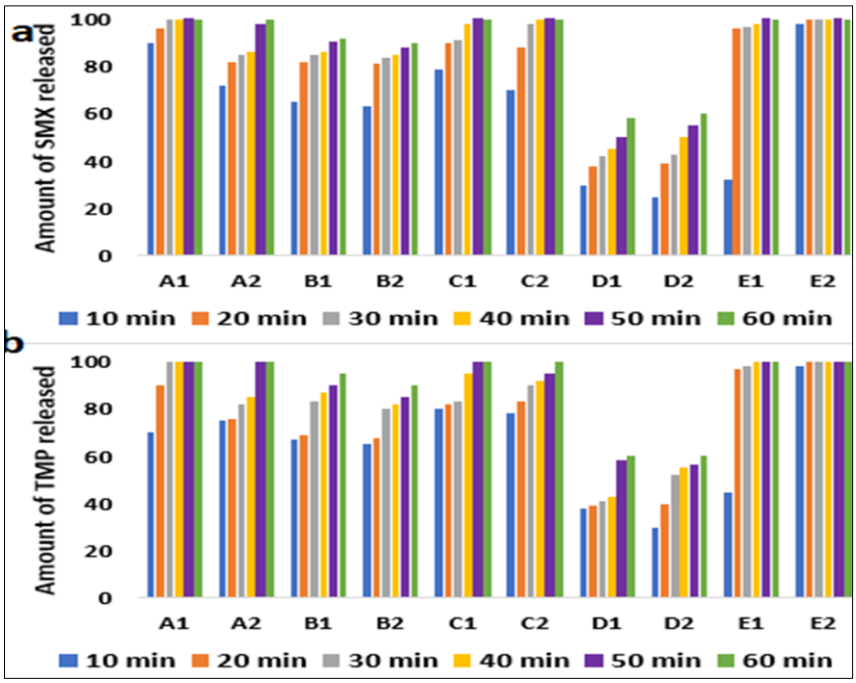


Figure 1 Dissolution profile of co-trimoxazole tablets in 0.1 M hydrochloric acid: Amount of (a) SMX, (b) TMP released over 60 min; with A1 to E2 shown on the x-axis depicting studied co-trimoxazole tablets and suspensions

Apart from the use of the first derivative UV Spectroscopy to evaluate the API content of co-trimoxazole tablets and suspensions, HPLC analysis of the co-trimoxazole products were also carried out to validate the reliability of the FDS method.

SMX was maximally absorbed at a wavelength of 255 nm while the wavelength of maximum absorption for TMP was 232 nm using first derivative UV spectroscopic method. Moreover, binary mixture of SMX and TMP exhibited distinct peaks, indicating that unknown samples containing SMX and TMP can be conveniently identified using the spectroscopic technique. The concentration ranges of SMX (15-35 mg/mL) and TMP (3-7 mg/mL) selected to prepare calibration curves were appropriate as there was linearity in the generated calibration curves, with a regression coefficient value of 0.999 and 0.996, respectively.

The USP recommends that the amount of SMX and TMP in co-trimoxazole tablets and suspensions should be between 90 % and 110 % [18]. The amount of SMX contained in the USP compliant co-trimoxazole tablets analysed using HPLC ranged from 99.2 to 107.5 % while that of TMP was between 90 to 104.4 %. On the other hand, SMX and TMP content of the tablet samples using FDS analysis was 97-109 % and 90-101.2 %, respectively. There was good correlation in the API content of the co-trimoxazole tablets evaluated using both analytical techniques. Overall, 60 % of the tested co-trimoxazole tablets passed the drug content test. This finding is in good agreement with the proportion of samples that passed the API content evaluation of co-trimoxazole tablet samples (59 %) procured from Katsina, Nigeria [17]. Nevertheless, co-trimoxazole tablets procured from Yemen and tested using HPLC revealed that all the locally manufactured and imported tablets exhibited satisfactory SMX (98.2 -103.8 %) and TMP (99.3 % - 103.8 %) content.

With the USP compliant co-trimoxazole suspension samples, the respective amount of SMX and TMP quantified using HPLC method of analysis was 90-108.8 % and 90.2-106.2 %. Also, UV analytical method revealed SMX content of the suspension formulation to be 97.5 %-102.4 % and all the suspension samples did not contain satisfactory level of TMP. On the other hand, 40 % of the co-trimoxazole suspensions passed the API content test based on HPLC analysis. The amount of co-trimoxazole suspension samples that passed the test was less than the percentage recorded at Nairobi, Kenya (70.6 %). This finding may be due to the disparity in the method of analyzing the API content of co-trimoxazole suspension as well as presence of substandard co-trimoxazole suspensions in the Lagos market.

There was disparity in the SMX and TMP content of the co-trimoxazole suspensions using UV and HPLC method of analysis. The generally low TMP content of the cotrimoxazole suspension may be due to its degradation in the solvent used for sample preparation as well as mobile phase, giving a false impression that all the tested suspension brands had failed the API content test. This finding was similar to that reported by Frimpong and co-workers, with all five

cotrimoxazole suspension samples procured from Ashanti, Ghana, and analysed using UV spectroscopy failing the drug content test [11], suggesting that the UV method of analysis may not be suitable to evaluate the API content of co-trimoxazole suspensions. The HPLC method may be more reliable than UV spectroscopic technique, to evaluate the API content of co-trimoxazole tablets and suspensions. Thus, a batch of tablet or suspension passed the drug content test if acceptable levels of SMX and TMP was detected in the drug product using FDS and/or HPLC technique.

Table 4 Drug content assay of co-trimoxazole tablets and suspensions using FDS and HPLC analysis

Samples	SMX		TMP		Remark based on HPLC and/or FDS data
	FDS assay (%)	HPLC assay (%)	FDS assay (%)	HPLC assay (%)	
A1	109.0±1.2	105.2±0.8	88.0±0.9	92.0±0.6	Passed
A2	106.0 ±1.6	107.5±1.0	101.0 ±1.2	103.2±0.8	Passed
B1	106.0±1.2	120.0±0.6 (F)	87.0±0.8 (F)	90.0±0.5	Failed
B2	102.0±1.4	105.0±0.5	90.0±0.9	91.0±0.6	Passed
C1	119.6±1.8 (F)	125.6±1.1 (F)	101.2±1.2	104.4±1.0	Failed
C2	116.8±1.0 (F)	120.8±1.0 (F)	97.9±0.9	101.2±0.9	Failed
D1	103.0±1.2	104.0±0.8	95.0±0.8	96.0±0.7	Passed
D2	98.0±1.2	100.2±0.7	88.0±0.7 (F)	88.4±0.6 (F)	Failed
E1	101.0±1.3	103.6±0.6	94.0±0.7	98.0±0.8	Passed
E2	97.0±1.0	99.2±0.4	93.0±0.7	97.2±1.1	Passed
F1	102.4±1.2	90.0±0.3	38.0±1.1 (F)	106.2±1.4	Passed
F2	72.0±1.2 (F)	107.6±0.5	37.0±1.0 (F)	97.7±1.1	Passed
G1	73.0±1.1 (F)	22.0±1.0 (F)	25.5±1.2 (F)	53.0±1.1 (F)	Failed
G2	72.0±1.2 (F)	24.0 ±1.0 (F)	24.0±1.2 (F)	55.0±1.1 (F)	Failed
H1	55.0±1.0 (F)	82.5±1.2 (F)	25.0±1.3 (F)	140.6±1.2 (F)	Failed
H2	72.0±1.2 (F)	88.0±1.1 (F)	13.0±1.0 (F)	142.6±1.2 (F)	Failed
I1	118.8±1.1 (F)	127.0±1.2 (F)	56.0±1.2 (F)	118.6±1.2 (F)	Failed
I2	114±1.2 (F)	120±1.2 (F)	38.0±1.0 (F)	115.5±1.2 (F)	Failed
J1	97.5±1.3	108.8±0.8	34.5±1.0 (F)	90.2±1.0	Passed
J2	116.6±1.2	114.6±0.5	46.0±1.1 (F)	90.8±1.0	Passed

Note: Pharmacopeial limit for co-trimoxazole tablets and suspension: 90-110 % of SMX and TMP; F = fail

There were statistical differences between the amount of SMX and TMP contained in co-trimoxazole tablets and suspensions using FDS and HPLC methods of analysis ($p < 0.5$). Nevertheless, both analytical techniques still revealed that comparable percentage of co-trimoxazole tablet samples passed the API content test.

Most of the USP non-compliant co-trimoxazole dosage forms did not contain acceptable level of sulphamethoxazole and trimethoprim. Nevertheless, there were samples that failed the test because they contained API levels that were greater than the upper limits set by USP. This intra-batch and inter-batch variations in API content may be due to poor adherence to good manufacturing practices and human errors during drug formulation (Table 4). The presence of extremely low and high levels of the API in the studied co-trimoxazole tablets and suspensions was not acceptable because the former may result in therapeutic failure and encourage antibacterial resistance while the latter may result in elevated side effects and adverse drug reactions.

4. Conclusion

Co-trimoxazole tablets and suspension brands procured from Lagos, Nigeria have been evaluated for their pharmacopeial and non-pharmacopoeial quality attributes. 70 % of the tested tablets exhibited satisfactory quality attributes in terms of tablet weight variation, hardness, and friability, disintegration and dissolution profile while all the suspension products displayed acceptable pH.

In addition, 60 % of the studied tablets contained appropriate amount of API specified by USP. On the other hand, lower proportion of the evaluated co-trimoxazole suspension brands (40 %) passed the API content evaluation using FDS and/or HPLC analysis whereas FDS analysis suggested that none of the suspension samples passed the API content assessment. The FDS spectroscopy may not be suitable for the quality assessment of co-trimoxazole suspension, and these formulations should be preferably analysed using HPLC method. Nevertheless, both FDS and HPLC analytical techniques were appropriate to analyse the API content of co-trimoxazole tablets. Thus, FDS technique may be considered to analyse co-trimoxazole tablets in resource-limited settings prevalent in the developing world. These findings have necessitated the need for stringent regulations to periodically evaluate and assure the quality of marketed antibiotic medicines, including co-trimoxazole products in order to ensure that safe, efficacious, and quality antibiotic medicines are available to the patients in order to prevent therapeutic failure or drug toxicity.

Compliance with ethical standards

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Authors' contributions

OC and BOS conceived and designed the study; OC carried out the experimental studies while OC and OMK analysed the data and drafted the manuscript; OMK and BOS revised the manuscript. All authors have read and approved the final manuscript.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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