QUALITY ASSESSMENT OF SOME BRANDS OF CO-TRIMOXAZOLE SUSPENSIONS AVAILABLE IN ILORIN, NIGERIA

BY

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ABSTRACT

Background: Fake and substandard drugs are becoming serious area of concern in our society. These drugs have led to therapeutic failure, adverse effects and microbial resistance to drugs. Therefore, there is the need for routine quality assessment of these drugs in the market to ascertain that they are stable, efficacious and safe for consumption.

Objective: This work was aimed at carrying out chemical and microbiological analysis on eleven brands of Co-trimoxazole suspensions sold in Ilorin, Kwara state and comparing results with a brand used as secondary standard using t-test analysis.

Methods: Chemical analysis was done using titrimetric methods to determine the percentage contents of the active components of the various samples of Co-trimoxazole suspension. Microbiological analysis was done using pour plate method to determine bacteria and fungi counts of samples. Analysis was done using SPSS computer software and p values less or equal 0.05 were considered statistically significant.

Results: One brand of the Co-trimoxazole suspension failed the chemical test using USP, 2006 specification with average trimethoprim content of 88.94 ± 0% while three brands failed using BP, 2013 specification. Two of these brands had sulfamethoxazole average contents of 109.19 ± 0.72% and 107.85 ± 0% in addition to the brand that failed the USP, 2006 specification. Sample t-test analysis showed that majority of samples varied significantly from sample used as standard.

Conclusion: Generally, over 70% of the various brands of Co-trimoxazole samples examined complied with official standards.

Keywords: Chemical analysis, Microbiological analysis, Co-trimoxazole, Ilorin.

INTRODUCTION

Counterfeit and substandard drugs are fast becoming a worldwide problem. Reports have shown that millions of dollars of counterfeit pharmaceuticals and personal care products move through various authorized and unauthorized channels. The World Health Organization (WHO), since 1984, has been collaborating and collating data related to counterfeit drugs. This has enabled the organization to develop a database on counterfeit drugs. The World Health Organization received 771 reports of counterfeit drugs from different countries between 1984 and 1999. Twenty-two percent of these reports came from industrialized countries, while the rest came from developing countries.[1] Forty six confidential reports of counterfeit drugs were received by WHO from 20 countries from January 1999 to October 2000. About 60% of these reports came from developing countries while the remaining 40% were reported by developed countries.[2] The US based centre for medicines in the public interest predicts that counterfeit drug sales will reach US$ 75 billion globally in 2010, an increase of more than 90% from 2005. Fifty nine cases of counterfeit drug cases were opened by the US Food and Drug Administration in 2011.[3] Nigeria has been stated to be the second largest producer of counterfeit medicines, accounting for about 23% of counterfeit medicines distributed worldwide after India which is thought to account for about 35%; and Pakistan, 13.3%.[4]

Drug availability, distribution and control are major concerns in health development as drugs constitute an important aspect of health development technology.[5] In Nigeria particularly since the mid 1980s shortages of drugs and other technologies have become pervasive threats to the medical care system.[6,7] In addition to this, the qualities of some of the available drugs are questionable. These questionable qualities can have negative impact on health which includes toxic effect to the body system, resistance of microorganisms to antibiotics and poor outcome on disease management. These drugs may contain harmful ingredients, little or no active ingredients, ingredients of substandard strength. Some might have lost potency due to improper storage, expired or produced under filthy conditions.[7]

It is the duty of healthcare providers especially the Pharmacists to ensure that drugs dispensed to patient are of good quality and will be able to produce the desired therapeutic effects. Therefore, there is the need to routinely determine the

quality of drugs in the market to ascertain that they are stable, efficacious and safe for consumption.

The United States Food and Drug Administration (USFDA) has identified some criteria measure to combat fake drugs which include securing the actual medicine and its packaging and increasing the vigilance and awareness of medicine counterfeiting.[8] This involves routine checks on the medicines we buy from Pharmacies and other medicine outlets. These routine checks involve among other things, chemical and microbiological analysis of available drugs. The chemical analysis of pharmaceuticals is a very important way of detecting the quality of a drug in the market. Most importantly, it aims at determining and ensuring that active ingredients claimed by manufacturers to be in a drug is actually what it is and this also tell us if the amount of these active ingredients can remain stable throughout the shelf life of such product. The microbiological analysis on the other hand gives information as to the types of microorganisms that are present in the formulation.

Studies carried out in Nigeria have shown that antibiotics are among the most prescribed drugs.[9-11] In addition Co-trimoxazole has been found to be among the commonly prescribed antibiotics.[12]

Co-trimoxazole is a combination of two antibiotics: sulfamethoxazole (sulfonamide) and trimethoprim (diaminopyrimidine). It is defined as a mixture of five parts of Sulfamethoxazole and one part of trimethoprim. It falls in the category of sulfonamide antibacterial. It is a chemo therapeutic agent exhibiting bactericidal activity against numerous gram positive and gram negative bacteria. It is indicated in urinary tract infections, respiratory tract infection including bronchitis, pneumonia, infections in cystic fibrosis, melloidosis, brucellosis, granuloma inguinlae, otitis media, skin infections. It is contra indicated in patient that are hypersensitive to sulfonamides or trimethoprim and patients with porphyria. Co-trimoxazole comes in form of tablets, oral suspension and injection.[13,14]

This work therefore focuses on chemical and microbiological analyses of liquid dosage form of co-trimoxazole which is commonly used in our society and on which limited studies have been carried out on. This in turn provides information on these drugs to the regulatory agencies and also ensures that these drugs being consumed by a large number of children in Nigeria are of the required standard and good

quality. In addition, this work was carried out in an area where previous and similar studies have not been done.

METHODS

1. COLLECTION OF SAMPLES

All samples of Co-trimoxazole suspensions available and obtainable in Ilorin, Kwara State as at the time this study was conducted were used. Samples were drawn from a total of fifteen (15) registered Pharmacy outlets. Eleven (11) different brands of Co-trimoxazole suspensions were obtained for analysis.

Septrin® suspension (Glaxo Smithkline Pharmaceuticals) was used as secondary standard. This was obtained directly from companies manufacturing them using the Company's sales representatives in Ilorin, Kwara State.

2. DETERMINATION OF PERCENTAGE CONTENT OF SULFAMETHOXAZOLE IN CO-TRIMOXAZOLE SUSPENSIONS

A total of eleven samples were analyzed. For each sample, a quantity of the suspension equivalent to 0.5g of sulfamethoxazole (12.5ml) was weighed using a pipette into 60ml of water and 10ml of hydrochloric acid. Mixture was mixed as completely as possible.

Then, 3g of Potassium bromide was added, cooled in ice and titrated slowly with 0.1M sodium nitrite, stirring constantly and determining the end point when the indicator (starch iodide paper) changed to a sharp blue black color.

Each ml of 0.1M Sodium nitrite VS is equivalent to 25.33mg of Sulfamethoxazole.[15]

This test was done in triplicate and the average value and standard deviation calculated and recorded for every sample.

3. DETERMINATION OF PERCENTAGE CONTENT OF TRIMETHOPRIM IN CO-TRIMOXAZOLE SUSPENSIONS

A total of eleven samples were analyzed. A quantity of the sample equivalent to 50mg of Trimethoprim (12.5ml) was accurately measured using a pipette. Then, 30ml of 0.1N Sodium hydroxide was added and then mixed to suspend. Extraction was done with four 50ml portions of Chloroform, washing each extract with the same two 10ml portions of 0.1N Sodium hydroxide. The combined Chloroform layer

was washed with water till washing was neutral. The Chloroform layer was evaporated to dryness. The residue was taken up by glacial acetic acid with the aid of gentle heat. This was titrated with 0.1N perchloric acid using crystal violet as indicator.

Each ml of 0.1N perchloric acid is equivalent to 0.02903g of Trimethoprim.[16]

This test was done in triplicate. The average value and standard deviation for each sample was calculated and recorded.

4. SCREENING FOR BACTERIAL CONTAMINATION OF THE PRODUCTS

The procedure for bacteria count involved the following:

Exactly 10ml of the sample (suspension) was pipetted into 90ml of Nutrient broth and mixed properly. Then 1ml of the resulting mixture was pipetted into sterile petri dishes in triplicates and 20mls of prepared sterile Nutrient Agar (NA) was also poured into each petri dish and cooled to 45^{o}C. The petri dishes were swirled for even distribution and allowed to solidify. The dishes were incubated at 37^{o}C for 48 hours. Bacteria count was estimated using a colony counter and then multiplied by the dilution factor to obtain the total count.

The procedure above was repeated in triplicate for every sample.

The average value and standard deviation for every sample was obtained and recorded.

5. SCREENING FOR FUNGAL CONTAMINATION OF THE PRODUCTS

Exactly 10ml of the sample was pipetted into 90ml of Saborand dextrose broth and mixed properly. Then, 1ml of the resulting mixture was pipetted into two sterile petri dishes and then 20ml of sterile Sabourand Dextrose Agar (SDA) was poured into each dish and swirled to allow for even distribution. The plates were incubated at 25^{o}C for five days. The fungal count was estimated using a colony counter and then multiplied by the dilution factor to obtain the total count. This procedure was repeated in triplicate for every sample of Co-trimoxazole suspension used for this study.

The average value and standard deviation for every sample was obtained and recorded.

6. STATISTICAL ANALYSIS

Average percentage content of active components in the samples was determined using arithmetic mean and standard deviation of the values obtained from performing the tests in triplicate.

Sample t- test analysis was used to determine statistical significance of average percentage content of the test samples with the standard samples. A computer software SPSS version 15 was used for analysis and p values less than 0.05 were considered statistically significant.

RESULTS

A total of eleven different brands of Co-trimoxazole suspensions found in Ilorin, Nigeria were used for this study.

Table 1 shows the label information of the Co-trimoxazole samples used for this study. All samples were suspensions and all samples claimed strength of 40mg of trimethoprim and 200mg of sulfamethoxazole per 5ml of the product. Also, all available samples were locally produced in Nigeria. None of the samples used for this study had expired as the time analysis was carried out on them. All the available brands had NAFDAC registration numbers.

TABLE 1: LABEL INFORMATION OF CO-TRIMOXAZOLE SUSPENSION SAMPLES

CODE	BATCH NUMBER	DATE OF MANUFACTURE	DATE OF EXPIRY	PLACE OF MANUFACTURE
ST01	3B802004	February, 2013	January, 2016	Ogun, Nigeria
ST02	LS213013	February, 2013	February, 2018	Lagos, Nigeria
ST03	TSS01	April, 2012	March, 2016	Ogun, Nigeria
ST04	TTS062	November, 2013	October, 2015	Ilorin, Nigeria
ST05	RPL0706	April, 2013	March, 2016	Ilorin, Nigeria
ST06	P11320	October, 2013	October, 2016	Lagos, Nigeria
ST07	034	August, 2013	August, 2016	Anambra, Nigeria
ST08	MB022	January, 2013	December, 2015	Lagos, Nigeria
ST09	0036	November, 2012	October, 2014	Enugu, Nigeria
ST10	20360100A	October, 2012	October, 2015	Lagos, Nigeria
ST11	PT043	September, 2013	September, 2015	Ilorin, Nigeria

Table 2 shows the result of the chemical analysis of trimethoprim and sulfamethoxazole contents of Co-timoxazole samples. According to the table, samples ST03 and ST05 had the lowest and highest content of trimethoprim with percentage content of 88.94 ± 0% and 106.37 ± 0.42% respectively while samples ST07 and ST01 had the lowest and highest content of Sulfamethoxazole with percentage content of 97.71 ± 0% and 109.19 ± 0.72% respectively.

TABLE 2: CHEMICAL ANALYSIS OF THE ACTIVE SUBSTANCES OF CO-TRIMOXAZOLE SAMPLES

CODE	AVERAGE PERCENTAGE CONTENT OF TRIMETHOPRIM ± SD	AVERAGE PERCENTAGE CONTENT OF SULFAMETHOXAZOLE ± SD
ST01	103.28 ± 0	109.19 ± 0.72
ST02	98.26 ± 0	106.39 ± 0
ST03	88.94 ± 0	98.06 ± 0.71
ST04	100.20 ± 0.41	105.04 ± 0
ST05	106.37 ± 0.42	106.63 ± 0.71
ST06	105.22 ± 0.42	107.85 ± 0
ST07	91.78 ± 0.76	97.71 ± 0
ST08	100.63 ± 0.41	103.45 ± 0.71
ST09	96.33 ± 0	100.16 ± 0.72
ST10	95.75 ± 0.41	99.30 ± 0
ST11	102.07 ± 0	102.23 ± 0.71
Septrin®	100.89 ± 0.41	101.85 ± 0

Table 3 shows the paired sample t-test analysis for percentage content of Trimethoprim using Septrin® suspension as secondary standard. Only two samples: ST04 and ST08 had p values of greater than 0.05 while all other samples had p values less than 0.05.

TABLE 3: PAIRED SAMPLE T-TEST ANALYSIS FOR PERCENTAGE CONTENT OF TRIMETHOPRIM USING SEPTRIN® SUSPENSION AS SECONDARY STANDARD

CODE	T VALUE	p VALUE
ST01	-67.400	0.000
ST02	53.694	0.000
ST03	278.860	0.000
ST04	1.522	0.268
ST05	-24.338	0.002
ST06	-23.057	0.002
ST07	41.316	0.001
ST08	-0.819	0.499
ST09	17.046	0.003

ST10	16.735	0.004
ST11	-6.602	0.022

Similarly, table 4 summarizes the result obtained when paired sample t-test analysis was carried out using percentage content of Sulphamethoxazole and Septrin® suspension as the secondary standard. Contrary to what was obtained using trimethoprim, five of the samples had p values less than 0.05 while the remaining six samples had p values greater than 0.05.

TABLE 4: PAIRED SAMPLE T-TEST ANALYSIS FOR PERCENTAGE CONTENT OF SULPHAMETHOXAZOLE USING SEPTRIN® SUSPENSION AS SECONDARY STANDARD

CODE	T VALUE	p VALUE
ST01	-20.000	0.002
ST02	-6.500	0.023
ST03	2.646	0.118
ST04	-10.000	0.010
ST05	-5.292	0.034
ST06	-6.425	0.023
ST07	4.000	0.057
ST08	-3.464	0.074
ST09	1.000	0.423
ST10	1.512	0.270
ST11	-1.000	0.423

No single sample of Co-trimoxazole suspension exhibited bacteria, fungal or pathogenic growth when samples were cultured.

DISCUSSION

This work focuses one of the commonest drug dosage forms usually purchased for paediatric use. In addition liquid dosage forms are commonly used for the production of pediatric products. The drug selected for this study was chosen because they are frequently administered to children. Co-trimoxazole suspension is also a commonly prescribed and purchased drug for respiratory tract infection in children in our society.

It was ensured that all samples purchased for this study were not expired. Drugs are expected to remain stable and intact throughout their shelf lives. During this period, the active components are supposed to remain within acceptable limit according to

specified standards and the preservatives within the drugs are expected to keep the drugs safe from harmful microorganisms. All the brands of Co-trimoxazole suspensions obtainable were all locally produced. The reason for non-availability of foreign brand of this drug may be as a result of prohibition placed on importation of some drugs into the country by NAFDAC and Nigeria Customs Services to promote local production of such drugs.[17]

Official monograph of BP, 2013 specify that the content of trimethoprim and sulfamethoxazole in co-trimoxazole suspensions should be between 92.5 and 107.5% while that of USP, 2006 specifies between 90 and 110%.[15,18] Out of the eleven brands of Co-trimoxazole suspension analyzed in this study, only one (ST03) failed the chemical analysis according to the USP monograph while three (ST01, ST03 and ST06) failed the chemical analysis according to the BP monograph (Table 2). ST03 brand failed the specification stated in the two official books. The 88.86% quantity of trimethoprim therein is below the specification in the two monographs although its Sulfamethoxazole content is within specification. Possible reasons for the low quantity of trimethoprim in sample ST03 may include introduction of sub optimal quantity into the bulk product which may be intentional, due to error during weighing or due to spillages and improper mixing of the bulk product which might result in higher quantity of the active ingredients in some parts of the product compared to other parts of the same product. The major danger here is that trimethoprim is an antibiotic and sub standard quantities of antibiotics in a preparation may give room for microbial resistance making it difficult to eradicate such organisms. Although the Sulfamethoxazole content of brands ST01 and ST06 passed using the USP monograph, they however failed the specification in the BP monograph as their percentage contents were higher than 107.5%. Therefore, the decision on whether the product has passed or failed will largely depend on the official book guide of the individual manufacturer. A lower quantity of antibiotics in a preparation which is out of specification is the common cause of bacterial resistance. A higher amount may only lead to untoward effects when used.

Aside the observations made above, it is good to note that majority of the co-trimoxazole suspensions obtained for analysis all passed the chemical analysis of their active components. This is a good development as it goes to show that in term of content of actives, majority of the drug found in the market are very reliable. The

result also tells us that despite the fact that the product is a suspension which has to be shaken to get an even mix, the average content and standard deviation suggest a good mix during compounding and filling of all the analyzed brands.

All samples of co-trimoxazole suspensions passed the microbiological analysis. None of them showed any bacteria, fungi or pathogenic growth when microbial analysis was carried out on them. This result is not surprising as co-trimoxazole on its own is a broad spectrum antibiotic which does not support growth of a wide variety of microorganisms within the product apart from the possible adherence to cGMP and addition of preservatives to the product. This showed that pediatric preparations of co-trimoxazole found in the market do not support growth to a very large extent.

A similar study carried out on co-trimoxazole suspensions in South Eastern Nigeria shows that all the analyzed products passed the chemical and microbiological tests carried out on them though microbial growths were observed in some of the samples. These growths were however within stated specification.[19] This result differs from that obtained from the present study.

Also, the result obtained from this study vary slightly with that obtained from a similar study conducted in Sagamu, Nigeria where three out of six samples of Co-trimoxazole analyzed failed to conform to official standards as regards the content of Sulfamethoxazole within the product.[20]

This work also compared results obtained using the titrimetric method with Septrin® suspension. Septrin® suspension is among the pioneer brand of co-trimoxazole suspension introduced into the drug market and also manufactured by a reputable Pharmaceutical company. The t-test analysis shows that only samples ST04 and ST08 were not statistically significant when compared with percentage content of Trimethoprim in the standard sample ($p>0.05$). This means that the percentage content of Trimethoprim in the secondary standard is only comparable to these two samples mentioned above. Other samples differed significantly. Sample ST03 which failed trimethoprim percentage content had one of the highest statistical difference when compared with the secondary standard ($t=278.860$; $p= 0.000$). On the other hand, t-test revealed that using the percentage content of Sulphamethoxazole, six of the samples are comparable to the standard samples ($p>0.05$). These are ST03,

ST07, ST08, ST09, ST10 and ST11. These samples did not statistically differ from the standard sample. ST03 which significantly differed statistically from the standard sample when tested for percentage content of trimethoprim was on the reverse side as regards sulphamethoxazole. Although, some of the test samples differed from the standard sample, this does not in any way infer that they failed the chemical tests carried out on them.

CONCLUSION

This work carried out chemical analysis using the titrimetric method and microbiological analysis using the pour plate method on eleven brands of co-trimoxazole suspensions. Based on BP 2013, three samples failed the chemical analysis (ST01, ST03 and ST06). However, only one brand of the samples (ST03) was found to be out of specification by failing the chemical analysis of trimethoprim based on USP 2006. Generally, majority (more than 70%) of the various brands of the two drugs under examination in Ilorin, Kwara state complied with official standards.

In addition, average percentage content of trimethoprim in nine brands and average percentage content of sulfamethoxazole in five brands of co-trimoxazole suspensions differed significantly from the standard sample when statistical test was done.

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