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Quality Evaluation of Some Brands of Amoxicillin Capsules Dispensed in Lagos State, Nigeria

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Abstract: *The efficacy of amoxicillin drug depends on the concentration of the active pharmaceutical ingredient, which is amoxicillin trihydrate. Amoxicillin also contains gelatin, carmoisine, quinoline yellow, titanium dioxide and iron oxide yellow. Some reports on the production and distribution of adulterated drugs have been recorded in several parts of Nigeria. This study investigated the quality of amoxicillin obtained from some patent drug stores in Lagos state, Nigeria. Analysis of weight uniformity, and disintegration were performed using standard methods. Determinations of the concentration of active pharmaceutical ingredient and heavy metals in amoxicillin samples were assayed through the employment of a high performance liquid chromatography (HPLC) and atomic absorption spectrophotometer (AAS) instrument respectively. Analytical results obtained, revealed that the weight of twenty samples of amoxicillin were within the compendia specification for uniformity of weight. The disintegration rates ranged from 4.01±0.3 to 7.13±0.2 minutes and were also within the permissible limits of ≤15 minutes. The percentage compositions of amoxicillin in the various analysed brands ranged from 91.2 to 100.4%, which are also within the recommended limits. The heavy metals concentrations in amoxicillin were within the permitted limit of ≤ 20 ppm. The results of this study revealed that the ten brands of amoxicillin samples analysed, conformed to the standard recommended limits and may not constitute medical threats beyond the expected range.*

Keywords: amoxicillin, concentration, disintegration, weight uniformity, heavy metal

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1.0 Introduction

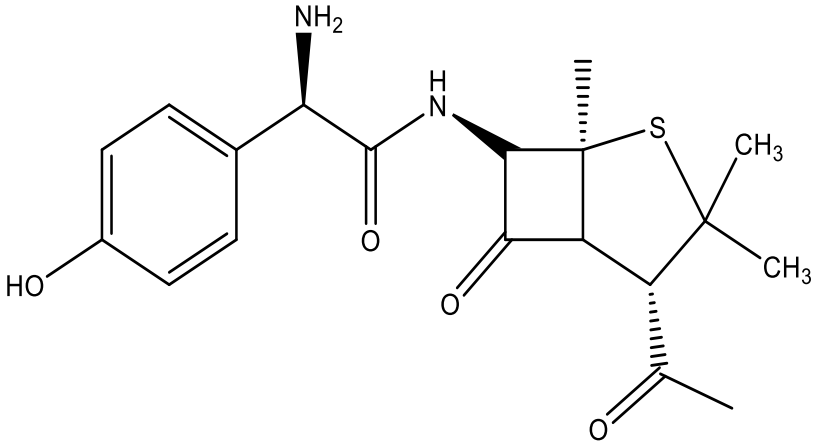
Substances in pharmaceutical drugs that are responsible for the beneficial or therapeutic health effects experienced by consumers are appropriately described as active pharmaceutical ingredients (WHO, 2006). Based on the United State Food and Drug Administration (US FDA, 2019) terminologies, an active pharmaceutical ingredients (APIs) should bring about 'pharmacological activity or other direct diagnostic effects, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the human body (Newton, 2006). Several studies

have correlated the purity and overall quality of active ingredients in drug products to the safety and efficacy of such drugs (David *et al*., 2018; Perur, 2018; Anah *et al*., 2019). Poorly manufactured and contaminated active ingredients have been linked to ill health and ultimate death (Mandal *et al*., 2012: Nduka *et al*., 2020). Drug formulations with low concentrations of the active ingredients are linked to bacterial resistance to antibiotics and associated impairment of curing rate and suppression of healing (Perur, 2018; Anah *et al*., 2019). Drug formulations with very high concentrations of the active ingredients are responsible for liver and kidney failure and eventually death in humans.

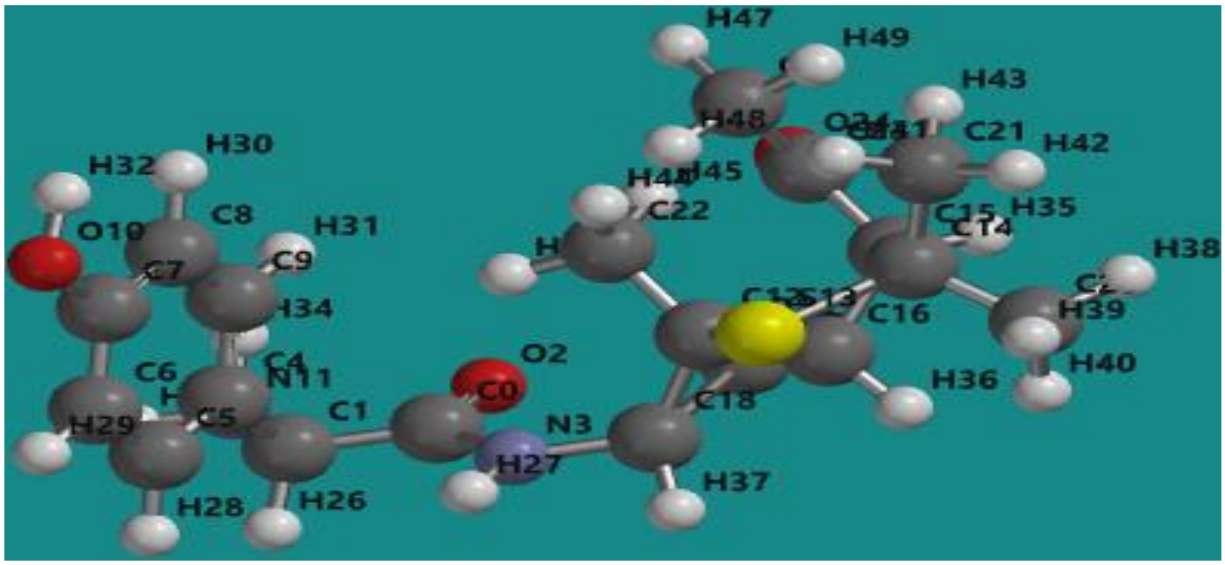
There is therefore a need for regulatory agencies and researchers to periodically determine the quantity of active pharmaceutical ingredients in drug formulations, thereby monitoring and exercising their regulatory functions. Standard chemically identical drug products or formulations should contain the same active pharmaceutical ingredients in the same strength and dosage. Furthermore, to ensure the safety and fitness of the drug for the intended purpose, the path of administration, quality, purity, disintegration rate, dissolution rate and content uniformity of the standard or generic drug products must be the same (Shah, 1994). Also, the concentration of binders, nature of lubricant, uncontrolled temperature and humidity at post storage stage may affect the quality of the active pharmaceutical ingredient and hence drug quality. In this study, a combination of pharmaceutical and chemical assay methods are adopted for the quantitative and qualitative determination of active pharmaceutical ingredients in generic amoxicillin drug samples. Amoxicillin is an antibacterial drug used in the treatment of several bacterial infections which includes acute otitis, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, salmonella infections, lyme disease, and chlamydia infection. Others are middle ear infections, skin infections and others (Scales, 2013; Davis, 2017). Amoxicillin is primarily administered orally in tablet form or hard gelatine capsule (ASHSP, 2015). The IUPAC name is given as [2S-[2α, 5α, 6β(S*)]]-6-[[Amino (4-hydroxyphenyl) acetyl] amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid trihydrate. It has a molecular formula: $C_{16}H_{19}N_3O_5S$ and the molecular weight: 365.40g/mol. The structure of amoxicillin is shown in Fig. 1a while Fig. 1 b shows the 3-D structure of the compound.

The application of amoxicillin is associated with some adverse reactions or side effects which include headache, diarrhea, stomach upset, skin rash, abdominal, taste sense and vaginal yeast infection. Also, there is a less common side effect which includes; allergic reactions like itching or hives, swelling of the face, lips, or tongue, breathing problems blistering, peeling, or loosening of the skin, including inside the mouth, trouble sleeping, seizures, dark urine, trouble passing urine, yellowing of the eye or skin, unusually weak and usually bleeding. (US FDA, 2019). A metallic chemical element that has a relatively high atomic weight and density at least five times much greater than water and is toxic at very low concentrations is known as heavy metal. Transition metal catalysts such as copper, nickel, rhodium, palladium, platinum, and chromium have been used in the process of manufacturing active pharmaceutical ingredients which therefore presents a possibility for the traces of these metals remaining after the purification of active pharmaceutical ingredients. Generally, the most common trace metals in drug formulations are arsenic, cadmium, lead and mercury, which are believed to have entered the manufacturing process from natural sources. (Schopp *et al*, 2015). Heavy metal poisoning is thought to occur through ingestion or faulty metabolic pathways (Nash *et al*, 2005). The purpose of the present study is to

access some physicochemical quality such as uniformity of weight and dissolution and the concentration of an active pharmaceutical ingredient in brands of amoxicillin using High Performance Liquid Chromatography (HPLC). The study also attempts to determine the levels of heavy metals in the brands of amoxicillin



2D STRUCTURE



3D STRUCTURE

Fig. 1: 2D and 3D structures of amoxicillin (2R)-N-((1S,4S,7S)-4-acetyl-1,3,3-trimethyl-6-oxo-2-thiabicyclo[3.2.0]heptan-7-yl)-2-amino-2-(4-hydroxyphenyl)acetamide)

2.0 Materials and Methods

2.1 Materials

Distilled water and de-ionized water were prepared by the use of milli-q50 water distiller system (USA) and deionizer system (DM-6000 Dual bed Deionizer, USA) correspondingly. Acetonitrile was of analytical grade and obtained from Sigma-Aldrich. Centrifuge, monobasic potassium phosphate, and potassium hydroxide were analytical grades purchased from Sigma-Aldrich. Amoxicillin reference standard (RS) was benevolently provided for this work from NAFDAC. Disintegration apparatus (YB/DT/01), analytical weighing balance (Metler Toledo, YE-AB-04), HPLC (Agilent 1260 infinity; version 2.1, Japan) and Atomic Absorption Spectrophotometer (Buck Scientific VPG 210)

constitute the leading instruments that were used for the analysis of amoxicillin. Ten (10) brands of amoxicillin capsules were randomly procured for the study from the popular distribution outlets in Idumota and Mushin, Lagos State. Brands of amoxicillin capsule were coded A_1- A_{10}.

2.2.1 Test for uniformity of weight

Twenty tablets of amoxicillin were randomly selected and the weight of each tablet was determined using analytical weighing balance (Metler Toledo, YE-AB-04). The average weight was then obtained and recorded.

2.2.2 Disintegration Test

Six tablets/capsules of amoxicillin were placed in the basket of the disintegration apparatus (YB/DT/01) to which some quantity of de-ionized water was added to submerge the tablets and maintained at 37ºC. The time taken for all the tablets to completely disintegrate was recorded minutes.

2.2.3 Analysis of amoxicillin by High performance liquid chromatographic method (HPLC)

2.2.3.1 Preparation of buffer solution, mobile phase and standard solution

A buffer solution was prepared by dissolving 6.8 g/L of monobasic potassium phosphate in water. The solution was adjusted with 45% potassium hydroxide to a pH of 5.0 ± 0.1. The mobile phase was prepared by adding acetonitrile to the buffer solution previously prepared above in the volume ratio of 1:24, whereas the standard solution was prepared by dissolving 1.2 mg/ml of amoxicillin reference standard (RS) in the buffer solution.

2.2.3.2 Preparation and analysis of sample solution

5 amoxicillin capsules were placed in a high glass blender jar containing buffer solution sufficient enough to make a concentration of 1 mg/ml of anhydrous amoxicillin. The supernatant mixture was then blended for 4 minutes and was allowed to stand for 5 min. About 20 ml of the mixture was taken and centrifuged for 3minutes. After which about 10 ml of the clear supernatant was passed through a filter to obtain a pure solution and the filtrate then subjected to HPLC analysis under the following chromatographic conditions, mode: liquid chromatography, detector: wavelength (UV) is 230 nm; column: 4 mm x 25 |cm; 10-µm packing L1, column temperature: 30 ºC, flow rate: 1.5 ml/min; injection volume: 10 µm. The concentration of the active ((amox) ingredient, was calculated using equation 1

$$Con(amox) = \left(\frac{R_u}{R_s}\right) \times \left(\frac{C_s}{C_u}\right) PF \times 100 \quad (1)$$

where: Ru is the peak response from the sample solution whereas Rs symbolized peak response from the standard solution. Cs represent the concentration of amoxicillin reference standard in standard solution (mg/ml) while Cu is the normal concentration of amoxicillin in the sample solution (mg/ml). P and F are the potencies of amoxicillin in reference standard (µg/mg) and the conversion factor (0.001 mg/µg) respectively.

2.2.4 Heavy metal determination

Analysis of heavy metals was done using atomic absorption spectrophotometer (Buck Scientific VPG 210). Serially diluted concentrations of stock solutions of the corresponding metal ions were used in the preparation of calibrated curves. Each concentration (mg/ml) of the metal ion in the analyte sample was obtained by graphical extrapolation from the calibration curve.

3.0 Results and Discussion

The chromatograms obtained from the assays of amoxicillin standard and sample are shown in Figs. 2 and 3 respectively.

In Table 1, results from weight uniformity test are recorded for the different samples of the amoxicillin that were analysed. The recorded results revealed that the mean weight uniformity investigated varied from one brand of amoxicillin to the other.

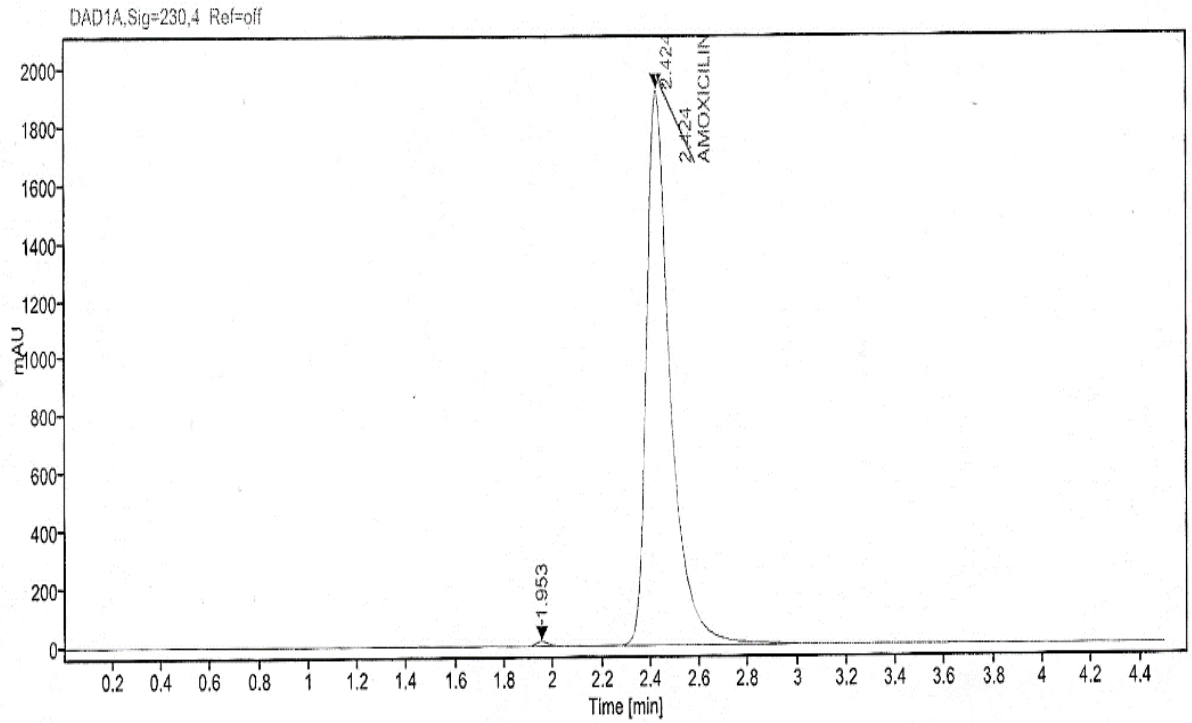


Fig. 2: A Chromatogram for amoxicillin standard

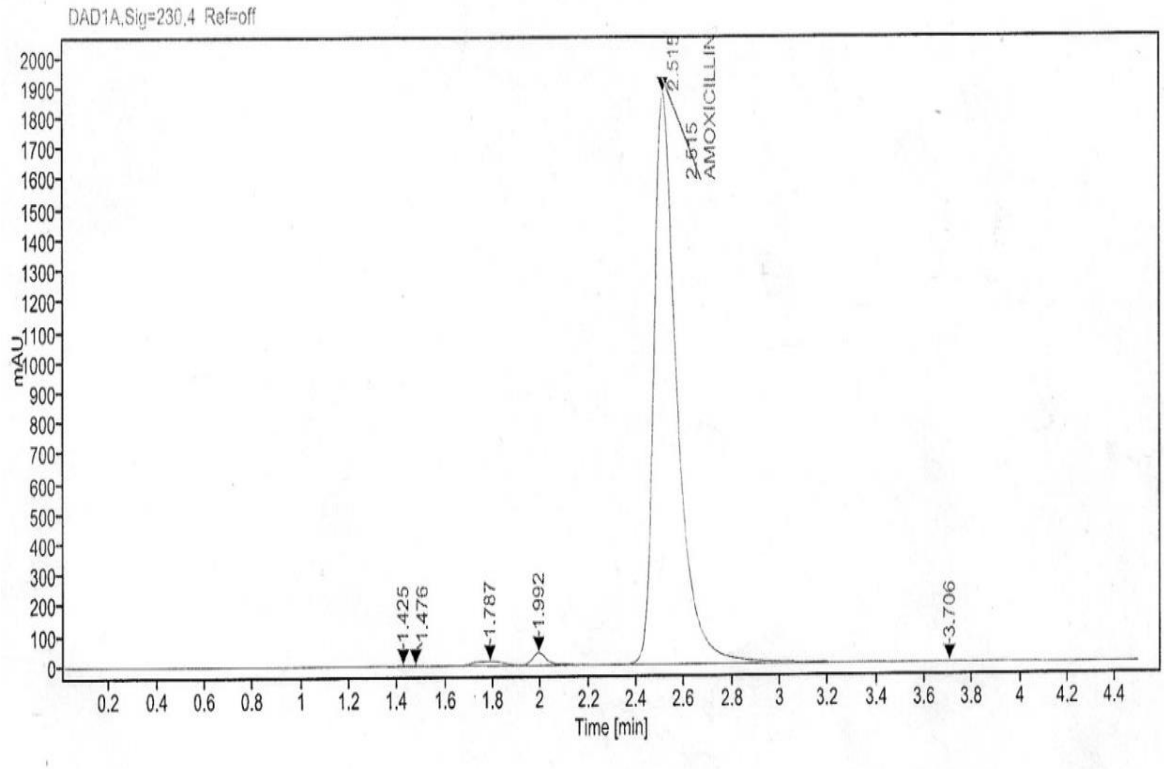


Fig. 3: A Chromatogram for amoxicillin sample

Table 1: Uniformity of weight for amoxicillin (mg)

Sample	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
1	526.7	603.2	563.5	537.9	574.8	546.8	613.2	553.5	557.0	564.4
2	574.3	621.6	561.1	560.1	535.6	594.1	631.4	554.1	580.1	525.1
3	552.8	613.5	539.6	557.4	572.6	572.3	633.6	527.2	577.3	562.4
4	590.1	604.4	554.8	561.1	588.2	610.1	616.4	544.8	541.1	578.1
5	506.1	651.7	577.0	562.3	573.1	526.2	663.7	566.0	542.3	583.4
6	537.9	582.9	568.1	559.8	591.5	559.1	593.2	558.1	579.5	601.3
7	522.3	593.4	558.0	559.1	585.1	544.3	603.3	548.4	549.2	565.4
8	563.8	641.6	565.6	587.7	573.2	583.4	653.6	555.8	597.5	583.2
9	577.3	604.3	546.5	558.5	589.7	593.3	614.5	536.4	568.5	599.6
10	531.7	583.8	536.6	562.4	555.2	551.5	594.6	526.3	572.5	565.3
11	542.9	573.5	553.9	578.2	563.6	563.7	583.4	543.6	589.2	583.6
12	567.3	597.0	534.9	581.9	556.2	583.3	606.1	524.3	591.7	578.2
13	523.6	609.5	543.1	564.3	575.6	543.5	619.6	533.2	584.3	595.1
14	533.1	642.2	558.8	549.5	583.8	554.1	652.4	546.4	558.5	593.6
15	531.1	571.6	512.5	559.1	571.7	553.1	581.4	502.5	579.8	591.7
16	553.6	623.2	569.6	584.1	573.9	576.3	643.3	557.6	594.1	594.0
17	551.6	597.4	554.8	561.2	548.1	571.7	607.2	544.4	581.8	568.3
18	516.4	614.3	527.6	555.7	583.4	537.4	624.1	516.6	575.4	593.6
19	560.8	602.1	563.7	557.2	542.3	582.5	613.1	543.7	586.2	565.3
20	532.8	642.1	548.9	591.6	595.2	552.5	652.3	536.5	601.5	589.2
mean	544.3	608.6	551.4	564.1	571.3	564.6	619.7	540.6	575.0	578.7
SD	21.88	22.54	15.56	13.09	16.31	21.27	23.37	15.40	17.12	17.59
Upper limit	610.0	676.2	598.1	603.4	620.2	628.4	689.8	586.8	626.4	631.5
Lower limit	478.7	541	504.7	524.8	522.4	500.8	549.6	494.4	523.6	525.9

Amoxicillin brands A_7 recorded higher values for content weight uniformity of 663.7 mg while A_1 and A_8 had lower weight uniformity values of 506.1 mg and 502.5 mg respectively. The values of standard deviation ranged from 13.09 to 23.37. Uniformity of content has significant in the quality control of drug formulation either in tablet or capsule form. Uniformity of content ensures that all drug formulations are within a tolerance of their average weight irrespective of the batch or time it was produced. It measures the quantity of active ingredients existing in the tablet or capsule and the quality of manufacturing practices. The mean content uniformity varied from 540.6 ± 15.40 to 619.7± 23.37 mg. The acceptable criteria for weight uniformity specify that not more than two weights out of twenty random weights should fall outside the boundaries of upper and lower limits (British Pharmacopoeia, 2015). Similarly, an alternative specification for uniformity of weight validates that out of twenty random weights, it must not exceed two weights that differ significantly from the mean weight by a value greater than 5% (British Pharmacopoeia, 2015). In this study, the results for content uniformity of weight were perfectly within the permissible compendia limits.

Values for the average disintegration time (in minute) of the investigated amoxicillin samples are recorded in Table 2. The results revealed that the disintegration rate varied from 4.01±0.3 to 7.13±0.2 minutes among the ten different brands of amoxicillin evaluated.

Table 2: Average disintegration for brands of amoxicillin capsules (mg)

Sample	**Average DT time (min.)**
A1	5.01±0.1
A2	7.02±0.1
A3	6.03±0.2
A4	6.05±0.1
A5	4.01±0.3
A6	5.50±0.1
A7	7.13±0.2
A8	6.01±0.1
A9	6.18±0.3
A10	4.11±0.2

Drug formulation must undergo disintegration before the active pharmaceutical ingredient in the drug can be released (Quodbach and Kleinebudde, 2016). The disintegration process of drugs enhances the dissolution and bioavailability of active pharmaceutical ingredients (Desai and Heng, 2016; Markl and Zeitler, 2017). The dissolution process increases the oral absorption of drugs and enhances therapeutic efficiency. The time taken for a tablet or capsule to disintegrate at body temperature (37 ^{o}C) must not exceed 30 minutes (US Pharmacopoeia, 2016). The maximum permissible time for disintegration for uncoated tablets and coated tablets were 15 minutes and 30 minutes respectively (British Pharmacopoeia, 2015). The results of amoxicillin disintegration were within the acceptable limits.

The composition of an active pharmaceutical ingredient in the ten brands of the amoxicillin formulation was analysed with the aid of high-performance liquid chromatography (HPLC). The concentration of amoxicillin is presented in Table 3. The results of the composition showed a noticeable difference in the concentration of active pharmaceutical ingredients in the brands of amoxicillin analysed. The British Pharmacopeia demands that the composition of amoxicillin should not be lower than 95% and not higher than 105% while the United State Pharmacopoeia recommended acceptable criteria that range from 90.0% - 110.0%. The mean concentration of amoxicillin ranged from 455.9 ± 0.10 mg to 501.8 ± 0.05mg while the label weight claim for all the ten brands of amoxicillin was 500 mg. The percentage composition ranged from 91.2 to 100.4%, indicating that amoxicillin content in the drug formulations are within the recommended specifications.

Table 3: Mean Composition by weight of amoxicillin

SAMPLE	**Mean Composition by weight (mg)**	**Label weight claim (mg)**	**Percentage composition (%)**
A1	501.8± 0.05	500	100.4
A2	498.4±0.01	500	99.7
A3	483.9±0.02	500	96.8
A4	489.4±0.03	500	97. 9
A5	455.9±0,10	500	91.2
A6	458.5±0.06	500	91.7
A7	471.8±0.20	500	94.4
A8	461.1±0.12	500	92.3
A9	493.0±0.02	500	98.6
A10	498.7±0.30	500	99.7

Also, measured concentrations of heavy metals in the amoxicillin samples (Table 4) were observed to vary markedly from one metal to the other, but with insignificant difference in levels of variation among the analysed metals.

Table 4. Heavy metal concentration in amoxicillin capsules (ppm)

Sample Code	Concentration of amoxicillin (ppm)				
	Cu	**Cd**	**Fe**	**Pb**	**Zn**
A_1	0.25±0.05	0.06±0.01	0.60±0.00	0.00	0.77±0.01
A_2	0.30±0.02	0.07±0.01	0.68±0.01	ND	1.02±0.03
A_3	0.19±0.05	0.06±0.01	0.65±0.01	ND	1.49±0.01
A_4	0.25±0.03	0.05±0.00	0.63±0.01	0.00	1.68±0.02
A_5	0.26±0.01	0.08±0.01	0.91±0.02	0.00	1.20±0.01
A_6	0.27±0.01	0.05±0.01	0.70±0.01	0.00	0.79±0.01
A_7	0.29±0.03	0.08±0.01	0.72±0.03	0.00	1.13±0.02
A_8	0.24±0.04	0.07±0.01	0.68±0.02	0.00	1.61±0.03
A_9	0.31±0.01	0.06±0.01	0.66±0.01	0.00	1.70±0.02
A_{10}	0.28±0.02	0.09±0.00	0.99±0.03	0.00	1.33±0.01
Mean	0.264±0.027	0.067±0.01	0.722±0.015	0.00	1.272±0.017

The concentration of copper (Cu) in the analysed samples ranged from 0.19 ± 0.05 to 0.31 ± 0.01 ppm with a mean value of 0.264 ± 0.027 ppm. Cadmium had a maximum concentration of 0.09 ± 0.00 ppm in sample A_{10} whereas minimum levels of 0.05 ± 0.01 ppm and 0.05 ± 0.00 ppm were recorded in samples A_6 and A_4 respectively. The concentrations of iron (Fe) and zinc (Zn) ranged from 0.60 ± 0.00 to 0.99 ± 0.03 ppm and 0.77 ± 0.01 to 1.70 ± 0.02 ppm respectively. However, the concentration of lead was below the detection limit. The allowable limit for heavy metals concentration in amoxicillin formulation is 20 ppm (British Pharmacopoeia, 2015; Kovaleva *et al*., 2018). Therefore, measured concentrations of these heavy metals were within the permissible limits.

4.0 Conclusion

Results of uniformity of weight and dissolution obtained from the ten brands of amoxicillin formulations were within the permissible international limits. The concentrations of active pharmaceutical ingredients and the heavy metals were also within the acceptable recommended limits and hence, the drug formulations are fit for consumption and to perform therapeutic functions efficiently. Regular monitoring of both locally manufactured and imported drugs are well advocated to circumvent incidents of poorly manufactured or substandard drugs from flooding our markets.

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Conflict of Interest

The authors declare no conflict of interest