

Evaluation of heavy metal impurities in metformin tablet brands marketed in Ilorin, Nigeria

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Abstract

The safety and quality of pharmaceutical products are important considerations for optimal therapeutic outcomes. Periodic assessment of heavy metals impurities in pharmaceutical products used for long-term treatment of chronic diseases is important to safeguard the health and well-being of patients. This study investigated the levels of manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in brands of metformin tablets commonly prescribed in Ilorin, Nigeria, for the management of diabetes. Five brands of commonly prescribed metformin tablets were randomly purchased from pharmacies in Ilorin, North-central Nigeria. Metal concentrations in the acid digested samples were profiled using Atomic Absorption Spectrophotometry (AAS) method. Method validation results showed excellent linearity ($r^2=0.9921$ (Mn), 0.9995 (Cu), 0.9998 (Zn), 0.9996(Cd), 0.9913(Pb)), with limits of detection (0.001, 0.001, 0.002, 0.002 and 0.025 mg/L for Mn, Cu, Zn, Cd and Pb respectively) and limits of quantification (0.003, 0.003, 0.006, 0.006 and 0.076 mg/L for Mn, Cu, Zn, Cd and Pb respectively) in the concentration ranges of 2– 10, 0.2–1.6, 0.2–1.6, 1–4 and 1–4 mg/L for Mn, Cu, Zn, Cd and Pb respectively. Mn, Cu, and Zn were detectable in varying concentrations in all the five brands of metformin evaluated in this study while none of the brands contained Cd and Pb at detectable levels. The levels of Mn, Cu and Zn ranged between 0.031-0.062, 0.085-0.112 and 0.040-0.130 mg/mL respectively across the brands. The highest concentrations of Mn, Cu and Zn were 0.062(0.001), 0.112(0.001) and 0.130(0.001) mg/L respectively. Similarly, maximum daily ingestible amounts for Mn, Cu and Zn ranges between 0.84-1.75, 2.17-3.03 and 6.12-20.7 μ g/day respectively, which were below permissible daily exposure (PDE). These results indicate that all the metformin brands evaluated could be safely administered orally to the patients without fear of any of the metal's toxicity consequences.

Keywords: Metformin tablets; heavy metals; permissible daily exposure

Introduction

The safety and quality of pharmaceutical products are important considerations to ensure an optimum therapeutic outcome. Of great importance are drug products used for the management of chronic disease conditions such as oral antidiabetic agents, antilipemic agents, nicotine replacement therapy among others. These types of drug products are required to be taken for a long period. Heavy metal impurities are difficult to eliminate from

drug products. For instance, they can be added deliberately as catalysts during the manufacturing of active pharmaceutical ingredients (API) and also gotten from raw materials (excipients) or reagents that are used in the manufacturing processes. Similarly, more of these impurities can contaminate the drug products during the formulation process through leaching as well as hard and ware from equipment or vessels used in the manufacturing process¹. For this reason, contaminated drug

products are potential sources through which heavy metals can enter the human body². Monitoring the levels of potentially toxic metals in drug products used for the treatment of chronic disease conditions such as diabetes is important because potentially toxic metals, such as Mn, Cu, Zn, Cd and Pb have serious health implications when accumulated above the tolerable amount in the body³⁻⁷. The presence of trace amounts of potentially toxic metals in drug products is of great public health importance and poses a serious threat to safety issues on drug products ingested into the body. Accumulation of these heavy metal impurities in the human body can result in serious health hazards⁸. Hence, the periodic monitoring of heavy metals impurities in drug products will help the manufacturers to take necessary precautionary measures during manufacturing of drug products. It also helps to provide safety awareness on the risk associated with the consumption of drug products contaminated with high content of heavy metals to the regulatory bodies and the public. Diabetes is a chronic disease condition that requires daily medication intake to maintain normal blood sugar for optimum health benefits. Metformin presented in figure 1 is the most commonly used antidiabetic medication in the management of this chronic disease.

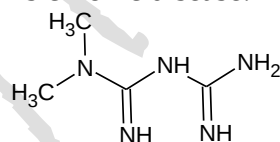


Figure 1: Chemical structure of metformin
Metformin is a biguanide used as a first-line medication for the treatment of type 2 diabetes^{9,10}. It decreases glucose production by the liver and increases insulin sensitivity to the body tissues⁹, through activation of adenosine monophosphate dependent (AMP) kinase in liver and muscle tissues. It also increases GDF15 secretion, with subsequent reduction in appetite and caloric intake¹¹⁻¹³. The fact that metformin helps in weight loss contributes to its choice as the first-line medication for managing overweight type 2 diabetes patients.

It was approved for use in France in 1957 and in the US in 1995^{9,14}, and listed as an essential medicine by WHO¹⁵. It is one of the most commonly prescribed medications for the treatment of diabetes in the US^{16,17} and is available in various forms in tablets of 500, 850 or 1000 mg, with a recommended regimen of a maximum of 3000 mg daily, taken in two to three divided doses. Metformin is available in extended-release formulations and in combinations with other antidiabetic drugs and is generally well tolerated with fewer side effects¹⁸. Seeing that metformin is a first-line medication in the treatment of diabetes, and a drug require to be taken for a long term, it is important to ensure its formulation is free from heavy metal impurities, hence, the present study aimed to determine the toxic trace contaminants of Mn, Cu, Zn, Cd and Pb in metformin tablet brands used in the management of diabetes using atomic absorption spectrophotometry and to compare the heavy metal contents obtained with predetermined oral permissible daily exposure (PDE) limits published by USP NF 2013¹⁹.

Materials and Methods

Reagents and instruments

Analytical grade nitric acid and perchloric acid were purchased from Merck Ltd, United Kingdom. Heavy metal reference standards were donated by the Central Research Laboratory, Obafemi Awolowo University while the heavy metal analysis was carried out using an Atomic Absorption Spectrophotometer (Analyst 400, Perkin Elmer AAS). Whatman ashless filter papers were used for the filtration of metal solutions. All glassware were Pyrex grade.

Collection of metformin tablet brands and heavy metal reference standards

Metformin tablet brands were collected in accordance with Medicine Quality Assessment Reporting Guidelines²⁰. Five commonly used brands of metformin tablets were randomly

purchased from prominent retail and wholesale pharmacies at different locations in Ilorin, North Central Nigeria. The samples were identified by the name of the company, batch number, National Agency for Food and Drug Administration and Control (NAFDAC) registration number, manufacturing and expiry date, batch number and country of manufacture. The samples were then coded as MET-A to MET-E. Heavy metal reference standards; lead (Pb), copper (Cu), cadmium (Cd), manganese (Mn) and zinc (Zn) were all obtained from Merck-KGaA (Darmstadt, Germany) and donated by the Central Research Laboratory (CRL), Ile-Ife, Nigeria for the study.

Pretreatment and Sterilization of Apparatus

All glassware and crucibles were soaked in 10% HNO₃ for 24 hours and washed with distilled water and then rinsed with de-ionized water before oven-dried at 105°C. Volumetric flask and measuring cylinder were dried at room temperature under control conditions to minimize contaminants.

Blank Determination

In blank determination, all the reagents used in the sample digestion was prepared in the same proportion and digested but without drug samples in triplicates. The values of metals obtained were subtracted from the ones of the digested samples.

Preparation of reference standards and calibration curves

Solutions of reference standards (100 mg/mL) were prepared by dissolving 1g of each reference standard for Mn, Cu, Zn, Cd and Pb in 10 mL of 1% nitric acid and further diluted with de-ionized water to obtain a working standard solution of 1 mg/mL. The working standard solution of each metal was used to prepare calibration standards for each metal. The calibration curve for each metal was prepared using five different concentrations

from serial dilution of working standard solutions of each metal and the absorbance of the metal solutions were taken at $\lambda_{\max}$ 279.48, 324.75, 213.86, 228.8 and 283.31 nm for Mn, Cu, Zn, Cd and Pb respectively. The nebulizer, atomizer and burner were flushed with de-ionized water before analysis of each sample. The functionality of the equipment was periodically monitored during the analysis²¹. The calibration curves for each metal were generated from absorbance readings plotted against the corresponding concentrations of each metal. Linearity was evaluated using the correlation coefficient (r^2) of the calibration curve for each metal. The sensitivity of the method was determined using a limit of detection (LOD) and limit of quantification (LOQ) based on the standard deviation of the y-intercept and the slope of the three independent calibration curves as specified by the International Council for Harmonization (ICH) guidelines²². LOD and LOQ for each metal were calculated as $3.3 \cdot \sigma/S$ and $10 \cdot \sigma/S$ respectively, where σ is the standard deviation of the y-intercept and S is the slope of the calibration curves for each metal studied.

Sample preparation and digestion

0.5g of the ground sample from each brand of metformin tablet was weighed into a 50 mL conical flask. 25 mL concentrated nitric acid and Perchloric acid (HNO₃ and HClO₄) in ratio 3:1 was added and was heated on a hot plate at a temperature of 115°C until it dissolves and gives a clear solution. The obtained solution was allowed to cool and then filtered into a 25 mL volumetric flask and made up to the 25 mL mark with de-ionized water. Some portion of the solution was taken for mineral composition using the atomic absorption spectrophotometric method²³.

Instrumentation and sample analysis

A validated atomic absorption spectrophotometer (AAS) with deuterium background correction was used for the analysis of Mn, Cu, Zn, Cd and Pb in metformin tablet brands. Argon gas was used

for flushing the furnace. The standards, blank and sample solutions were analyzed for each metal using appropriate hollow cathode lamps after sample digestion. The lamp current flow (A) was 20(Mn), 15(Cu), 15(Zn), 4(Cd) and 10(Pb). The wavelengths ($\lambda_{\max}$) used for this analysis were 279.48, 324.75, 213.86, 228.8 and 283.31nm for Mn, Cu, Zn, Cd and Pb respectively. The concentration of each metal in the samples was extrapolated from the corresponding standard calibration curves.

Maximum daily ingestible amounts

The maximum amount ingestible on daily basis for Mn, Cu, Zn, Cd, and Pb were calculated based on the maximum recommended daily dose (3g) for metformin oral tablet (6 tablets of 500mg each) by computing the amount of each metal obtained in a tablet and multiply by 6. The metal intakes were compared with the

established permissible daily exposure (PDE) limits^{24,25}.

Statistical analysis:

The statistical analysis of the results was performed on Microsoft Excel 2016 (Microsoft Inc, Office 2016). Data were presented in mean, standard deviation (SD), regression coefficient (r^2) and relative standard deviation (RSD).

Results

The descriptive characteristics of the various brands of metformin tablets evaluated showed that four of the five selected brands were manufactured in Nigeria and the fifth was imported into the country from Spain. All were NAFDAC registered and were all within the expiration period as at the time of analysis (Table 1).

Table 1: Descriptive characteristics of various brands of metformin tablets

Brand code	Average weight of tablet (g)	Manufacturer location	NAFDAC Reg. No	Batch/ Lot No	Mfg. Date	Expiry Date
MET-A	0.5354	Spain	04-6233	E206479	12/2020	11/2025
MET-B	0.5309	Nigeria	B4-0076	21002	03/2021	02/2024
MET-C	0.6731	Nigeria	A4-2278	FVU2004	12/2020	11/2023
MET-D	0.5629	Nigeria	04-0810	CA10584	01/10/2020	30/09/2023
MET-E	0.5095	Nigeria	A4-4310	AC21051	07/2021	06/2023

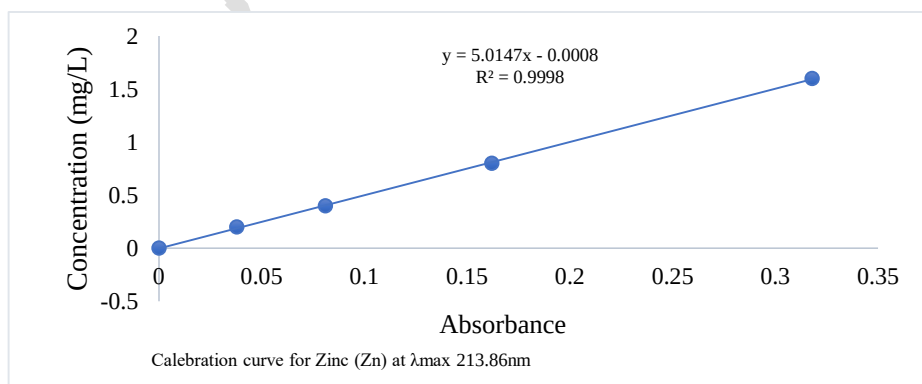
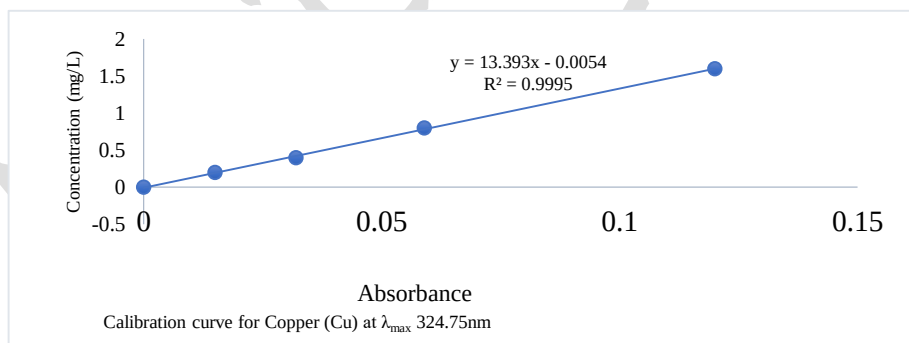
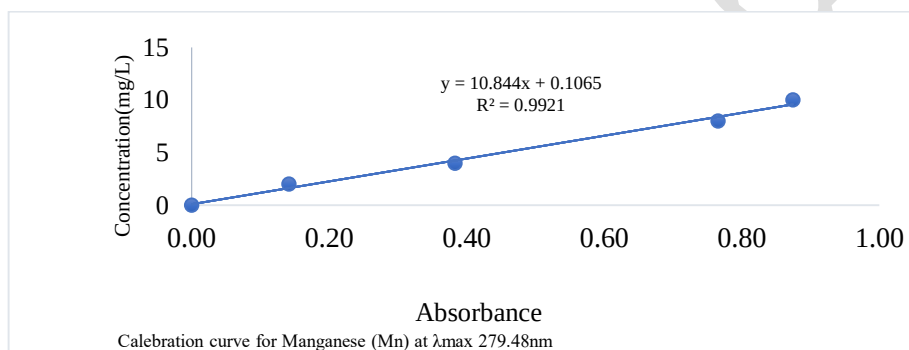
The five brands of metformin tablets were quantified for Mn, Zn, Cu, Cd and Pb using the atomic absorption spectrophotometry (AAS) method. Calibration curves for reference standards of the respective metals were linear over the concentration ranges of 0.2-1.6, 0.2-1.6, 1-4, 1-4 mg/L for Cu, Zn, Cd and Pb respectively (Figure 2 & Table 2). The regression coefficients (r^2) of the calibration curves were 0.9921, 0.9995, 0.9998, 0.9996 and 0.9913 for Mn, Cu, Zn, Cd and Pb respectively (Table 2). The LOD were 0.001, 0.001, 0.002, 0.002 and 0.025 mg/L and LOQ

were 0.003, 0.003, 0.006, 0.006 and 0.076 mg/mL for Mn, Cu, Zn, Cd and Pb respectively (Table 2).

Metal concentrations found in the five brands of metformin tablets are presented in Table 4. Mn, Cu, and Zn showed varying amounts across the metformin tablet brands while none of the brands contained Cd and Pb at detectable levels. The levels of Mn, Cu and Zn ranged between 0.031 to 0.062, 0.085 to 0.112 and 0.040 to 0.130 mg/mL respectively across the various brands (Table 3).

The highest concentrations of Mn, Cu and Zn were 0.062 (0.001), 0.112 (0.001) and 0.130 (0.001) mg/L respectively and they were all observed in MET-B. Similarly, maximum daily ingestible amounts were also computed but the results showed that all the five elements have the amount far below the PDE limits, with the levels for Mn, Cu and Zn ranging between 0.84 to 1.75, 2.17 to 3.03 and 6.12 to 20.7 µg/day

respectively across the various brands (Table 4). The highest daily ingested amount for Mn, Cu and Zn were 10.5 (MET-C), 18.18 (MET-C), and 20.7 (MET-B) µg/day respectively. The PDE limits for Cu and Zn are 3000 and 13000 µg/day as established by International Conference on Harmonization and 2500 µg/day for Mn by European Medicines Agency (EMA)^{24,25}.



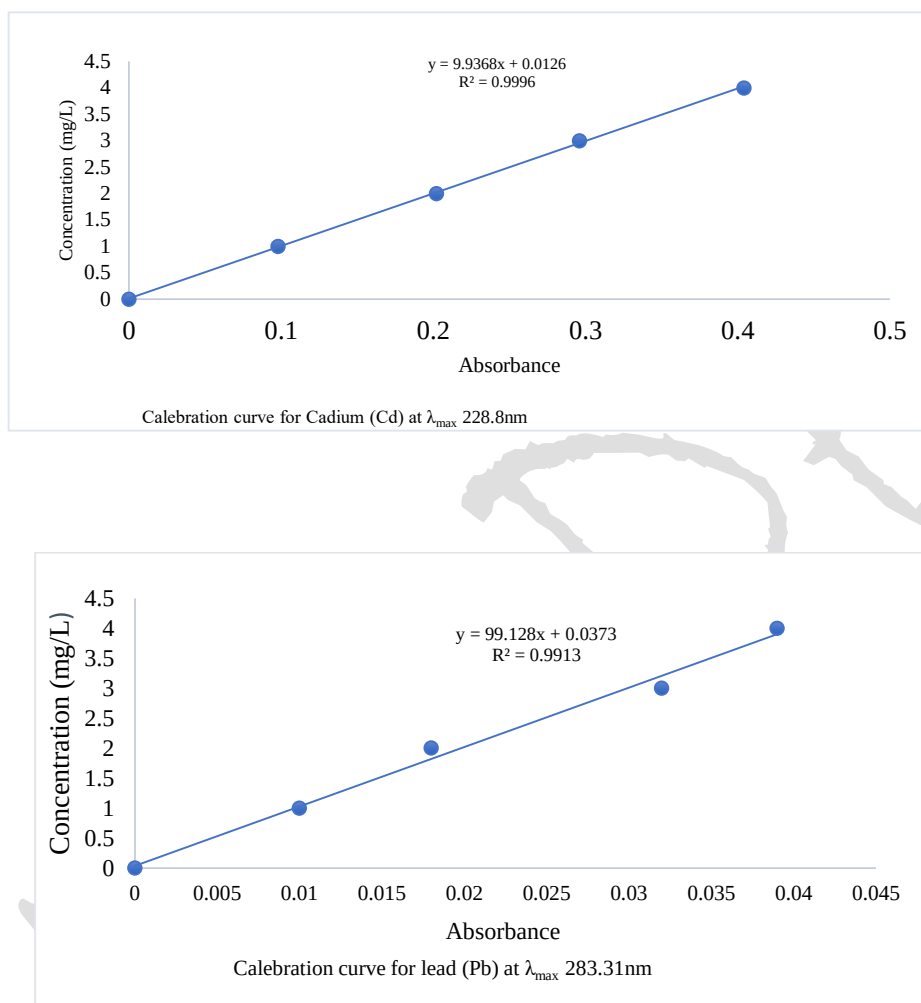


Figure 2: Standard celebration curves for Mn, Cu, Zn, Cd and Pb at their respective $\lambda_{\max}$

Table 2: Optical characteristics of the calibration curve

Parameters	Mn	Cu	Zn	Cd	Pb
Detection wavelength ($\lambda_{\max}$) (nm)	279.48	324.75	213.86	228.80	283.31
Current I (A)	20	15	15	4	10
Linearity range (mg/L)	2-10	0.2-1.6	0.2-1.6	1- 4	1- 4
Regression equation	10.844x + 0.1065	13.393x - 0.0054	5.0147x - 0.0008	9.9368x + 0.0126	99.128x + 0.0373
Correlation coefficient (r^2)	0.9921	0.9995	0.9998	0.9996	0.9913
Limit of detection (LOD) (mg/L)	0.001	0.001	0.002	0.002	0.025
Limit of quantification (LOQ) (mg/L)	0.003	0.003	0.006	0.006	0.076

Table 3: Concentrations of heavy metals present in 0.5g powder of each Metformin tablets brands

Sample Code	Mn(mg/L)	Cu(mg/L)	Zn(mg/L)	Cd(mg/L)	Pb(mg/L)
MET-A	0.031(0.000)	0.099(0.001)	0.044 (0.001)	BDL	BDL
MET-B	0.062(0.001)	0.112(0.001)	0.130 (0.001)	BDL	BDL
MET-C	0.052(0.001)	0.090 (0.000)	0.073 (0.001)	BDL	BDL
MET-D	0.042(0.001)	0.085 (0.001)	0.047 (0.001)	BDL	BDL
MET-E	0.041(0.000)	0.085 (0.001)	0.040 (0.001)	BDL	BDL

BDL: Below detection level; results are mean $\pm$ SD; $n=3$; SD: standard deviation; Mn: manganese, Cu: copper, Zn: Zinc, Pb: lead, Cd: cadmium.

Table 4: Maximum daily ingestible amounts of heavy metals from each metformin tablet brands

Metals	Amount	Metformin tablet brands					Permissible Daily Exposure (PDE) (µg/day) limit
		MET-A	MET-B	MET-C	MET-D	MET-E	
	Daily dose of metformin (g)*	3	3	3	3	3	
Mn	Amount found (µg/tablet)	0.84	1.65	1.75	1.18	1.05	2500 ^b
	Maximum amount ingested (µg/day)	5.04	9.9	10.5	7.08	6.3	
Cu	Amount found (µg/tablet)	2.65	2.97	3.03	2.39	2.17	3000 ^a
	Maximum amount ingested (µg/day)	15.90	17.82	18.18	14.34	13.02	
Zn	Amount found (µg/tablet)	1.18	3.45	2.46	1.32	1.02	13000 ^b
	Maximum amount ingested (µg/day)	7.8	20.7	14.74	7.92	6.12	
Cd	Amount found (µg/tablet)	BDL	BDL	BDL	BDL	BDL	5 ^a
	Maximum amount ingested (µg/day)	BDL	BDL	BDL	BDL	BDL	
Pb	Amount found (µg/tablet)	BDL	BDL	BDL	BDL	BDL	5 ^a
	Maximum amount ingested (µg/day)	BDL	BDL	BDL	BDL	BDL	

*Maximum recommended daily dose for Metformin oral tablet (6 tablets of 500 mg each), BDL: Below detection level, results are mean $\pm$ SD, $n=3$, SD: standard deviation, Mn: manganese, Cu: copper, Zn: Zinc, Pb: lead, Cd: cadmium. a: Limits of elemental impurities as per International Conference on Harmonization (ICH) b: Classification as per European Medicines Agency (EMA).

Discussion

The quality and safety of pharmaceutical products are fundamental issues that should not be compromised before, during and after manufacturing of pharmaceutical products. Hence, the provision for quality control measures at every stage of manufacturing. Of important concern is the safety assessment of heavy metal impurities in the finished pharmaceutical products. In practical terms, it is not possible to eliminate heavy metal impurities from pharmaceutical products because of many sources through which these impurities get into pharmaceutical products²⁶. For instance, during synthesis of active pharmaceutical ingredients (API), heavy metals can be added intentionally as catalysts,

or accidentally, which can contaminate the finished products²⁶⁻²⁹. Excipient, utilities (water), packaging containers as well as manufacturing equipment are other sources by which pharmaceutical products get contaminated with heavy metals²⁵. However, permissible daily exposure (PDE) limits have been established in official books such as USP and BP. PDE limits for heavy metals impurities in pharmaceutical products and individual components are well spelt out by International Conference on Harmonization (ICH) and European Medicines Agency (EMA) based on the level of safety assessment carried out on potential elemental impurities as previously classified^{24,25}.

The presence of heavy metal impurities poses a great health hazard especially with pharmaceuticals used to treat chronic diseases such as metformin. In this case, gradual accumulation of heavy metal contaminants in the body is inevitable which can further worsen a patient deplorable health condition. Since pharmaceutical products are not always free from certain level of toxic heavy metal contaminants during manufacturing processes, the present study evaluated the amount of Mn, Zn, Cu, Cd and Pb present in five commonly prescribed brands of metformin tablets in Nigeria. This will help to ensure the safety of both local and imported metformin tablet brands. Three of the five selected brands were manufactured by multinational pharmaceutical companies in their Nigeria-based manufacturing plants, one was imported and the fifth was manufactured by an indigenous pharmaceutical manufacturing company.

The validation parameters indicated that the assay method was precise, accurate and robust. Findings from the study revealed that Cd and Pb were absent or below the detectable limits in all the five brands of metformin tablets evaluated, while Mn, Cu and Zn were present in varying amounts. However, the maximum amounts of Mn, Cu and Zn ingested per day were below the estimated daily intakes of these metals as recommended by ICH and EMA^{24,25}. This shows that the daily oral intake of these metals upon administration of any of the five brands of metformin tablets to patients will be safe from toxicities associated with Mn, Cu and Zn. Mn is an essential nutrient for intracellular activities and serve as an important cofactor for varieties of enzyme such as glutamine synthase and arginase. It helps to activate the enzyme prolidase responsible for proline production, an amino acid requires for the formation of collagen that aids the wound healing process³. A high amount of Mn in the body can cause manganese toxicity such as neurological

disorders with symptoms like tremors, difficulty in walking and facial muscle spasms^{23,30}. Since the levels of Mn in the present study are lower than PDE limits, patients on these brands of metformin tablets are expected to be free from Mn toxicity. Instead, it will contribute to the daily intake of Mn to maintain normal body functions.

Similarly, Cu is an essential element that plays significant roles in many physiological and metabolic processes, serving as a co-factor and helping to activate a number of enzymes involve in metabolic processes such as haemoglobin formation, detoxification, metabolism of biomolecules, fetal and infant growth and development, brain development and function as well as immune function^{31,32}. High Cu levels in the body have been reported to cause skin infection, irritation of the respiratory tract; reproductive, developmental and neurological disorders as well as carcinogenicity, mutagenicity and hepatotoxicity^{33,34}. It is therefore important to ensure that pharmaceutical products are regularly checked to ensure the elemental impurities such as Cu are within the PDE limits.

In addition, toxicity due to excessive exposure to Zn has been reported to be uncommon. However, excessive intake of Zn interferes with Cu absorption in the gastrointestinal tract (GIT) which result in Cu deficiency. Excessive exposure to Zn is therefore characterized by the symptoms of low levels of Cu in the body, which include decline in body immune function and high-density lipoproteins³⁵. Prolong exposure to Cd causes cancer and organ system toxicity³⁶, while Pb toxicity affects almost all body functions which may result to death³⁷.

Generally, it is important to note that the present study is limited by a few numbers of heavy metals that were evaluated in the study. This leaves other potentially toxic metals such as mercury and arsenic³⁸ unevaluated. The limited numbers of heavy metals evaluated

was partly due to a limited number of reference standards, equipment and resources available for the analysis of elemental impurities. Hence, the present study could not make a general statement regarding the safety of the five brands of metformin tablets as regards all potential high-risk elemental impurities. Notwithstanding, the findings show that the levels of Mn, Cu, Zn, Cd and Pb in the five brands evaluated are far below the PDE limits and thus, safe within the scope of the present study. Regular evaluation of elemental impurities in pharmaceutical products is recommended, especially for drugs required to be taken for a very long period of time. This will help to safeguard the health and well-being of patients who are on such long-term medications.

Conclusion

The five brands of metformin tablets studied revealed a quantifiable amount of Mn, Cu and Zn which are lower than PDE limits, while Cd and Pb were below the detectable levels. This poses no health risk associated with the studied heavy metals on the patients who are on the long-term prescription of these brands of metformin tablets. However, complete analysis of all potentially toxic metal impurities is recommended to ensure the safety and quality of pharmaceutical products, to safeguard the health and well-being of Nigerians.

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