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Research Article

DETERMINATION OF ELEMENTAL IMPURITIES IN SOME COMMERCIAL PAEDIATRIC AND ADULT FORMULATIONS OF ARTEMETHER – LUMEFANTRINE IN THE NIGERIAN MARKET BY ATOMIC ABSORPTION SPECTROSCOPY

Sunday O. Awofisayo*[1], Augustine O. Okhamafe[2], Mathew I. Arhewoh[2]

[1]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, P. O. Box 4274, Uyo Akwa Ibom State, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, 300001, Nigeria

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*Corresponding author
Sunday O. Awofisayo

Email:
jdjide@yahoo.com

ABSTRACT

The composition of elemental impurities in paediatric powder for suspension (PPS) and double strength tablet (DST) commercial anti-malarial formulations of artemether-lumefantrine (AL) were determined. Six randomly selected PPS and DST products from a list of marketed products were purchased from a registered Pharmacy outlet and analyzed using flame atomic absorption spectrophotometer. The heavy metal levels were compared with the official limits for permitted daily exposure. The paediatric and adult products of AL presented the same types and number of elements with a total of 10 metals and levels ranging from 0.001-0.016 and 0.001-0.017ppm for the PPS and DST formulation, respectively. One of the DST and PPS products had significantly higher levels of cadmium, copper, chromium, nickel and cobalt compared with their respective formulation types ($p<0.05$). There was significant difference in the levels of chromium, nickel and cobalt but no difference in the levels of cadmium, lead, zinc and arsenic when DST and PPS products were compared ($p<0.05$). The drug products presented elemental oral exposure significantly lower than the permitted daily exposure. The levels of elemental impurities are satisfactory with respect to safety requirements. These impurities may adversely affect drug stability, shelf-life and bioequivalence of the products.

Key words: Elemental impurities, Permitted Daily Exposure, Artemether-lumefantrine, Paediatric Powder for Suspension, Double Strength Tablets

INTRODUCTION

Metals and metalloid impurities are an increasing focus in the pharmaceutical industry and for regulatory agencies anticipating high standards of quality control/quality assurance (QC/QA). An impurity in a drug substance as defined by the International Conference on Harmonization (ICH) Guidelines as any component of the drug substance that is not the active chemical entity (*i.e.,* the drug substance) or its supportive ingredients (*i.e.,* excipients) that affects the purity of the active ingredients or the drug substance (ICH, 2000). Metal particles are common contaminants in the drug manufacturing process (ICH, 1997; 2000; Ahuja and Alsante, 2003). The understanding of the levels of impurities in finished products or their components and their impact with respect to safety requires process knowledge (USP, 1990; 2014; ICH, 2006; Goroj, 2006).

The recent recalls of several prescription only medicine (POM) and over-the counter (OTC) medications due to metal contaminations and the possible toxicity or stability implications show that more efforts need to be in place during drug production, aimed at preventing metallic contaminants from reaching the public (Connors *et al*, 1986; Ahuja, 1998; Nageswara and Nagaraju, 2003; Lewen *et al*., 2004; Venkatesan and Valliapan, 2014).

The most common physical signs of presence of contaminants are the appearances of slimy metal flakes, or dark brittle particles ranging in colour from red to orange to brown (Kyle *et al.,* 2006). Techniques such as inductively coupled plasma-mass spectrometry (ICP-MS), inductively coupled plasma optical emission

spectrometry (employing arc and spark excitation), X-ray fluorescent (XRF) spectrometry, atomic absorption spectroscopy (AAS) and stationary or mobile metal analyzer. AAS is a spectro-analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiations of free atoms in the gaseous state (Muller *et al*., 1981; Lin *et al*., 2010).

The toxicity of trace metals like cadmium, mercury, arsenic and lead known as the "Big four analyte" is well known but their limits in pharmaceutical products have not been clearly defined (Wang *et al*., 2000). Some drug products have been analyzed for metallic impurities including some commercial aspirin formulations (Al-Taeb *et al.,* 2015). Artemether-lumefantrine (AL) is a fixed-dose artemisinin combination therapy (ACT) that was recommended by World Health Organization (WHO) for the treatment of uncomplicated malaria (White, 1998). Some reports on the content of antimalarial drugs including AL drug products have featured drug content levels below official specifications along with stability problems (Reddy and Banerji, 2012; Sridhar *et al.,* 2010). The significance of inherent metallic impurities in drug stability along with the efficacy and toxicity implications cannot be over-emphasized.

The aim of this work was to determine the levels of elemental impurities present in the selected AL drug products in order to ascertain if they meet requirements for daily exposure.

MATERIAL AND METHODS

Reagents

Hydrogen peroxide and Aqua-regia (mixture of concentrated hydrochloric acid: nitric acid 3:1) were analytical grade, products of Sigma Chemical Co., Germany. AL paediatric powder for suspension (PPS) and adult double strength tablets (DST) were purchased locally in registered pharmaceutical outlets in Uyo, Southern Nigeria.

Product sample selection

The analyzed drug products were collected between May and June 2013 from a list of National Agency for Food and Drug Administration and Control (NAFDAC) approved DST and PPS product in the study area. Table I presents the detail of the selected products. All the products were within their shelf life.

Sample preparation

A weight of 0.5g of powdered sample was taken from the PPS and 2 crushed DST formulations with the digital balance (Ohaus, China) to give an equivalent weight of 57.14 and 345.86mg of artemether and lumefantrine, respectively. The samples were prepared for close vessel digestion by adding 2mL of aqua-regia in a tight fitting reagent bottle. This was allowed to stand for 2min, after which 5mL of hydrogen peroxide was added with the lid of the reagent bottle loosely covered allowing the escape of gas while the samples get fully digested (Amadi *et al.,* 2012).

Spectroscopic determination

The digested samples were placed in a 3D double beam optics flame atomic absorption spectrophotometer (Schimadzu AA7000, Japan) and the results determined by a computerized software package (Aspect CS software, India) that measures the elemental amounts using an in-built cookbook method for all elements and a pre - defined calibration curve.

Statistical analysis

Mean values and standard deviations were computed using Minitab for Windows, Minitab Release 11.21, Minitab Inc., UK. Further data analyses were carried out using student's t-test and significant differences were observed at $p<0.05$.

RESULT AND DISCUSSION

A total of 10 elemental impurities were detected in the products with only 7 considered to be of pharmaceutical relevance and their levels further analyzed for safety and stability considerations. Figure 1 gives a chart of the frequency of occurrence of the elements in the selected products. The elements occurred almost to the same extent in all the sampled products of the two formulations. Table II and III present the elemental impurities and levels in the DST and PPS products, respectively. Table IV gives the comparison of the DST and PPS drug products with respect to the differences in the levels of the various impurities.

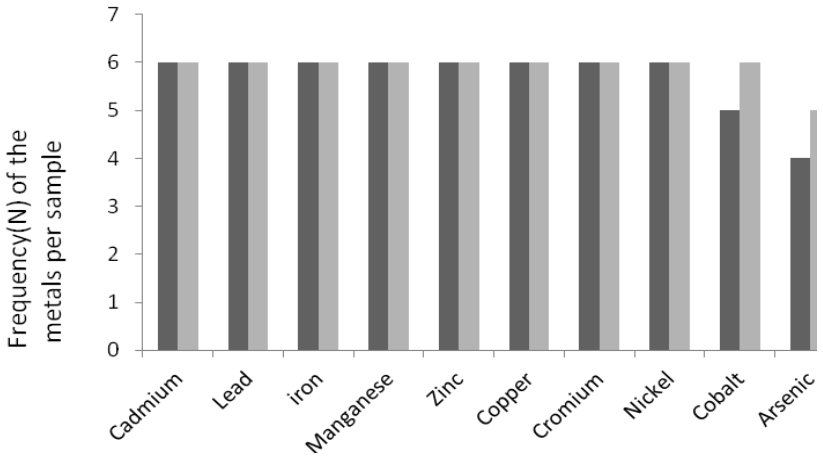

Figure 1. Frequency (N) of occurrence of individual metals in PPS(■) and DST(□) formulations.

Table I. Details of the brands of antimalarial PPS and DST studied

Product Code	Origin	Man-Exp Date	Batch Number	NAFDAC Reg. no
AL1	India	07/2012-06/2015	LD337	04-9927
AL2	India	08/2012-07/2015	ATMH-0014	A4-3489
AL3	Nigeria	02/2013-01/2015	3B76-0002	A4-5641
AL4	India	12/2012-11/2015	113	A4-3799
AL5	China	04/2013-04/2016	130409	A4-1225
AL6	Nigeria	07/2012-08/2015	04	A4-3935
ALA	China	10/2012-10/2015	HD121012	A4-1244
ALB	China	04/2012-04/2015	120430	A4-7402
ALC	China	06/2012-06/2015	120614	A4-1896
ALD	India	04/2012-03/2015	FD017	A4-6335
ALE	Nigeria	09/2013-09/2015	A41303	A4-4550
ALF	China	07/2013-07/2016	130701	A4-3646

Man- Exp Date- Manufacturing-Expiry Date; NAFDAC - National Agency for Food and Drug Administration and Control

There were no significant differences in the levels of cadmium, lead, zinc and arsenic in the two types of formulations as observed in the sampled products but significant differences in the levels of chromium, nickel and cobalt.

The choice for the use of flame AAS for the elemental analysis of the products was premised on the sensitivity, selectivity (measures individual metals concentration) and robustness of the equipment in detecting and quantifying the elemental impurities in trace amounts. The software employed in the analysis aided in the identification and quantification of the metal impurities. All the investigated products have essentially the same composition of elemental impurities (Figure 1). This indicated that the impurities are possibly inherent in the raw materials (*i.e.*, the bulk powder products). Most elemental impurities are introduced to bulk powder for drug production via the catalysts or other reagents employed in drug synthesis.

The different sourcing of active ingredients/excipients in the bulk powder for the preparation of these products during manufacture and interactions between formulation and packaging materials are ready routes of metallic contamination.

Table II. The concentration ± SD of metals present in the DST products

Metal	Concentration of elements in drug product(x 10^{-3} ppm, mean±SD, n = 3)					
	AL1	AL2	AL3	AL4	AL5	AL6
Cd	6.0±0.1	9.0±0.1	7.0±0.1	1.0±0.1	8.0±0.1	11.0±0.1
Pb	6.0±1.7	4.0±0.3	4.0±0.4	2.0±0.3	7.0±0.6	8.0±0.4
Zn	11.0±2.1	9.0±4.2	10.0±0.2	10.0±1.7	14.0±4.1	18.0±0.9
Cr	11.0±0.3	15.0±0.3	14.0±0.8	12.0±0.8	16.0±2.1	21.0±1.1
Ni	3.0±0.3	3.0±0.3	4.0±0.8	5.0±0.8	4.0±2.1	11.0±1.1
Co	3.0±0	2.0±0	3.0±0	3.0±0	3.0±0	6.0±0
As	0	1.0±0	2.0±0	2.0±0	1.0±0	1.0±0

Table III. Mean content (± SD) of metals in PPS products

Metal	Concentration of elements in drug product (x10^{-3}ppm, mean±SD, n = 3)					
	ALA	ALB	ALC	ALD	ALE	ALF
Cd	8.0±0.9	4.0±0.5	4.0±1.0	4.0±0.7	5.0±0.9	3..0±0.9
Pb	7.0±0.1	4.0±0.1	2.0±0.4	4.0±0.1	3.0±0.8	4.0±0.3
Zn	14.0±0.5	8.0±0.2	6.0±1.6	8.0±1.2	6.0±1.1	10.0±3.8
Cr	16.0±0.2	5.0±0.5	8.0±1.0	7.0±0.7	6.0±0.9	5.0±0.9
Ni	3.0±0.1	2.0±0.5	2.0±1.0	3.0±0.7	1.0±0.9	2.0±0.9
Co	3.0±0.9	0	1.0±0.1	2.0±0.7	2.0±0.9	1.0±0.9
As	1.0±0.9	1.0±0.5	1.0±0.1	0	1.0±0.9	0

Table IV Comparison of the elemental load in the two formulations (values are Mean ±SD)

Metal	Concentration of elements x10^{-3} ppm, mean±SD		
	DST	PPS	*P*-value
Cd	7.0±3.4	4.7±1.8	0.180
Pb	5.2 ± 2.2	4.0±1.7	0.330
Zn	15.0 ±3.5	8.7±3.0	0.110
Cr	14.8 ±3.6	8.7±4.0	0.012
Ni	5.0 ±2.6	2.2±0.8	0.077
Co	3.3 ±1.4	1.5±1.1	0.028
As	1.2 ±0.8	0.7±0.5	0.220

Traces of inorganic impurities can reduce drug stability and shelf life. This may be a reason for bioinequivalency of generic products (BP, 2001; ICH, 2006; Hoerle *et al.,* 1992). Products AL6 and ALA among the adult and paediatric products, respectively, had significantly higher levels of chromium, cobalt, cadmium and copper than the other products still indicating the source differences. Products AL1, ALD and ALF had no arsenic as elemental impurity compared with other products. The presence of metals such as arsenic, cobalt and lead in antimalarial product that are taken frequently especially in malaria endemic environment may pose some long term health risks.

Elements such as lead and cadmium are cumulative toxins. Infants and young children on the PPS may be particularly sensitive to the toxic effects of these metals because they tend to absorb a higher fraction of an oral dose. The developing body system (*i.e.*, the nervous system in children) may be more sensitive to the metals compared to mature system.

All the products had statistically lower oral exposure levels when compared with the permitted daily exposure, this therefore follows

that the safety of the products is established with respect to the USP chapter <232> (Venkatessan and Valliapan, 2014). Some earlier published works on artemether and lumefantrine have reported low chemical content of artemether and/or lumefantrine in generic products (Awofisayo *et al*., 2010). This has been explained based on the instability of artemether and/or lumefantrine or deliberate adulteration of the products. The role of these inherent elemental constituents may be investigated with respect to the low chemical contents of artemether and lumefantrine in their formulations.

CONCLUSION

The PPS and DST products contained the highlighted elements in amounts below the official limits with respect to patients' safety. The levels of contamination were however not sufficient to raise alarm with respect to the daily intake based on the two times dosing regimen of AL. The level of elemental content may, however, be contributory to stability issues that have been well reported about this ACT combination.

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