Heavy metal contamination of paediatric paracetamol and ascorbic acid drug products in South-West, Nigeria

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ABSTRACT

Background: Water is a primary contributor to human populations' heavy metals exposure and industrial contamination of products. Consequently, paediatric medications because of their high-water constitution can contribute significantly to intake of heavy metals in excess of allowable limits. There is therefore a need for effective preventive and control strategies. Unfortunately, studies investigating heavy metal content in paediatric formulations in Nigeria are scarce and often limited in the range of elements assayed.

Objectives: To evaluate elemental impurities in the two most frequently administered paediatric medications-paracetamol and ascorbic acid marketed in South-west Nigeria.

Methods: Thirteen paediatric syrup brands were used for the study. Sample pretreatment involved dry ashing followed by digestion using concentrated aqua regia (nitric acid:hydrochloric acid, 3:1). Chromium, lead, copper, cadmium, zinc, nickel, cobalt and manganese were assayed with the atomic absorption spectrophotometer with the limit of detection set at 0.001.

Results: The most abundant metal ions present in all the formulations were chromium (1.16-1290.2 mg/) and nickel (2.37-1289.0 mg/L). Cadmium was detected at low concentration in only two of the brands while lead was detected in three brands at concentrations ranging from 0.09 - 0.12 mg/L. The calculated expected daily exposures of lead in the three brands were in excess of the permissible daily exposure for oral drug products.
Conclusion: Some of the paracetamol and vitamin C syrups sold in the South-West of Nigeria are contaminated with cadmium, nickel and lead.

Keywords: *Elemental impurity, Heavy metal poisoning, Paediatric formulations, Paracetamol, Ascorbic acid*

Contamination par des métaux lourds de paracétamol pédiatrique et des médicaments à base d'acide ascorbique au sud-ouest du Nigéria

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RESUME

Contexte: L'eau est l'un des principaux facteurs qui contribuent à l'exposition des populations humaines aux métaux lourds et à la contamination industrielle des produits.Par conséquent, les médicaments pédiatriques, en raison de leur forte teneur en eau, peuvent contribuer de manière significative à la consommation de métaux lourds au-delà les limites autorisées. Des stratégies efficaces de prévention et de contrôle sont donc nécessaires. Malheureusement, les études portant sur la teneur en métaux lourds dans les formulations pédiatriques au Nigéria sont rares et souvent limitées dans la gamme des éléments testés.

Objectifs: Évaluer les impuretés élémentaires dans les deux médicaments pédiatriques les plus fréquemment administrés: le paracétamol et l'acide ascorbique commercialisés au sud-ouest du Nigéria.

Méthodes: Treize marques de sirop pédiatriques ont été utilisées pour cette étude. Le prétraitement de l'échantillon impliquait une incinération à sec suivie d'une digestion avec de l'eau régale concentrée (acide nitrique: acide chlorhydrique, 3: 1). Le chrome, le plomb, le cuivre, le cadmium, le zinc, le nickel, le cobalt et le manganèse ont été dosés avec le spectrophotomètre d'absorption atomique avec la limite de détection fixée à 0,001.

Résultats: Les ions métalliques les plus abondants présents dans toutes les formulations étaient le chrome (1,16-1290,2 mg/) et le nickel (2,37-1289,0 mg/L). Le cadmium a été détecté à faible concentration dans seulement deux des marques, tandis que le plomb a été détecté dans trois marques à des concentrations allant de 0,09 à 0,12 mg/L. Les expositions quotidiennes escomptées calculées de plomb dans les trois marques étaient supérieures à l'exposition quotidienne permise pour les médicaments par voie orale.

Conclusion: Certains sirops de paracétamol et de vitamine C vendus dans le sud-ouest du Nigéria sont contaminés par le cadmium, le nickel et le plomb.

Mots-clés:*Impureté élémentaire, Empoisonnement par les métaux lourds, formulations pédiatriques, paracétamol, acide ascorbique*

INTRODUCTION

Heavy metals have been variously defined as elements with a high atomic weight and specific gravity in excess of 5.0. However, the term *heavy metal* has recently been expanded to include metals and semimetals with potential human and environmental toxicity.[1, 2] Heavy metal poisoning is the accumulation of heavy metals in toxic amounts in the soft tissues of the body. Although considered to be few and far in between, incidences of heavy metal poisoning might not be as rare as the instances of its misdiagnosis.[3]

Apart from the more familiar acute iron and lead toxicity, symptoms of heavy metal poisoning (which can result in significant morbidity and mortality) are more likely to be erroneously attributed to other causes by physicians in emergencies.[4] The symptoms vary with the metal involved, the level and duration of exposure and the bioavailability which in turn is dependent on physicochemical properties including temperature, phase association, chelating ability, lipid solubility and partition coefficient.[2] For instance, although copper is one of those regarded as essential (the others are magnesium, iron, chromium, zinc, cobalt, manganese, molybdenum, selenium) as it is a required co-factor for several enzymes including those involved in haemoglobin formation, catecholamine biosynthesis etc.;[5] it can cause extensive cellular damage in humans leading to Wilson's disease when present in sufficiently high amounts.[2] Metal ions can also bind to oligonucleotides, oligopeptides, induce DNA conformational changes and have been implicated in carcinogenicity and apoptosis.[6-8]

The severity of the toxicity of heavy metal poisoning is more pronounced *in utero* and in infancy as a result of the rapidly dividing cells, maturing internal organs and metabolic pathways.[9] A recent study showed significantly heightened body levels of toxic metals in autistic children as well as a correlation between the levels and the severity of the autism.[10] Prevalence of developmental and behavioural problems have also been linked to bioaccumulation of the metals arising from proximity to dumps and point sources of environmental release[11, 12] and the intake of foods and consumables containing heavy metals in excess of acceptable limits.[13-15] Paediatric medications can contribute significantly to this as they are mostly liquid dosage forms with water as the primary solvent and the largest constituent of such formulations. Water is a primary contributor to human populations' heavy metals exposure and industrial contamination of products.[16] This is of particular concern in Nigeria with inadequate public water facilities and where many drug manufacturing companies privately source for water for the preparation of liquid dosage forms and for other unit operations.[17] Studies investigating heavy metal content in paediatric formulations in Nigeria are scarce and often limited in the range of elements assayed.[18] Among these formulations are paracetamol and vitamin C syrups which are the two most frequently administered medications in the treatment of common ailments as malaria, cough and generally as a supplement.[19] Both drugs are also available on over-the-counter demands.

The objective of this study was therefore to evaluate the presence of lead, copper, chromium, cadmium, zinc, nickel, cobalt and manganese in paracetamol and vitamin C syrups sold in the market.

MATERIALS AND METHODS

Equipment

Atomic absorption spectrophotometer (Perkin Elmer A Analyst 200 Ayer Rajah, Singapore), Mettler analytical balance (Ohaus Pioneer Montreal, Canada), precoated TLC plates (Merck, Darmstadt, Germany), TLC tanks, crucibles, Electric hot plate (Gallenkamp London, UK), thermostated oven (Gallenkamp London, UK)

Materials and reagents

Different brands of un-expired paracetamol and vitamin C syrups were sourced from patent medicine stores and pharmaceutical shops in Abeokuta, Lagos and Ibadan. In all, 13 brands (8 brands of paracetamol and 5 brands of ascorbic acid syrups) were identified and purchased for this study. The five ascorbic acid formulations are subsequently referred to in this text as CA, CB, CC, CD and CE while the paracetamol syrups are code-named PA, PB, PC, PD, PE, PF, PG and PH. All samples were analysed while still within their shelf lives.

All reagents used were of analytical grade. These included 1000 mg/L stock solutions of the metals all from Inorganic ventures USA, silver nitrate, HCl, HNO_3 (BDH UK), methanol, ethanol (Sigma Aldrich, Germany), chloroform, glacial acetic acid, toluene, acetone (BDH, UK). A 0.1 M silver nitrate solution as well as 2 M solution of nitric acid were prepared as specified in the British Pharmacopoeia.[20]

Drug sampling technique

On a cross-sectional basis, eight and five brands of paracetamol and vitamin C syrups respectively were purchased in September, 2016 from patent medicine and pharmaceutical stores by one of the investigators (MOI)

with the assistance of a guide. The cities were chosen being the most populous in South-west Nigeria and were therefore envisaged may provide an adequate reflection of the situation in the region. No particular sampling technique was adopted other than the buyers posing as regular customers to purchase the medication. The different brands of the two drugs were sourced from drug stores wherever they were available until saturation was reached, i.e., no new brands were obtained from further visits to stores. In all, about twenty stores were visited before saturation was observed. Telephone calls to drug stores other than those visited were also made to confirm the unavailability of additional brands.

Identification tests

Thin layer chromatography:

Methanolic solutions (0.5 mg/mL) of the ascorbic acid formulations and ascorbic acid reference substance were spotted on the same precoated silica gel GF_{254} plate and developed using normal phase mobile solvent ethanol:water (6:1) as specified for ascorbic acid formulations.[21] A reversed phase thin layer chromatographic analysis was also carried out using liquid paraffin-coated silica gel plates and methanol:water (4:6) solvent system. The identification of the active ingredient in the five ascorbic acid formulations was confirmed by visualization of the developed plates under UV at 254 nm and daylight.

The presence of paracetamol in the eight commercially sourced paracetamol syrups was also investigated by spotting paracetamol reference substance and the methanolic solutions of the paracetamol syrups on the same silica gel plate. A solvent system comprising chloroform:glacial acetic acid:toluene:acetone (13:0.1:2:5) on precoated silica gel GF_{254} as well as a mobile phase system of methanol:water (4:6) on liquid paraffin-impregnated plates were used to develop the chromatograms for the normal and reversed phase TLC analyses respectively. The identification of the active ingredient in the eight paracetamol formulations was confirmed by visualization of the developed plates under UV at 254nm and daylight.

Basic test

The chemical test for the identification of ascorbic acid in the ascorbic acid syrups was carried out by adding 0.2 mL of 2 M nitric acid and 0.2 mL of 0.1 M silver nitrate solutions to a volume of the formulation in each case that is equivalent to 50 mg ascorbic acid.[20]

Preparation of calibration curves

Standard solutions from each of the metal stock solution of 1000 mg/L were prepared by appropriate dilution with deionised water. The ranges in mg/L of the calibration curves were 0-2.5 for manganese, 0-1.6 for zinc, 0-2.0 for cadmium, 0-4.0 for nickel, 0-6.0 for copper, 0-10.0 for lead and 0-5.0 for cobalt and chromium. The solutions were aspirated directly into the flame and the absorbance recorded using water as the blank. After each solution, the nebulizer, atomizer and burner were flushed with deionised water.[22]

Pretreatment of samples

The crucible was decontaminated by a process of sequential cleaning in a series of solutions namely lab-grade detergent solution and deionized water rinses, 6 M HCl solution and then 7.5 M HNO_3 solution and ultra-pure water rinses. Thereafter, labware was air dried in a laminar air flow-exhausting hood. Sample preparation was by dry ashing method which involved the addition of 30 mL of each syrup sample into the crucible, heating on a hot plate at 200 ^oC for 45 minutes, followed by further heating in a furnace at 500 ^oC at atmospheric pressure until the volume was reduced to near dryness.[18,22]

Digestion was done by the addition of 10 mL conc. aqua regia ($HCl:HNO_3$;3:1) after which it was heated to dryness before 30 mL deionized water was added. The mixture was thoroughly stirred, filtered before the volume was made up to 100 mL in a volumetric flask. Chromium, lead, copper, cadmium, zinc, nickel, cobalt and manganese were assayed with the atomic absorption spectrophotometer with the limit of detection set at 0.001.[18,22]

RESULTS

Identification tests

The presence of ascorbic acid in the five vitamin C brands was established with the basic spot test, which yielded a stable grey precipitate with the five formulations, and thin layer chromatography. The R_f values for all the vitamin C syrups and the chemical reference substance were 0.74 in ethanol:water (6:1) and 0.78 in methanol:water (4:6).

Identical R_f values of 0.38 and 0.40 in chloroform:glacial acetic acid:toluene:acetone (13:0.1:2:5) and methanol:water (4:6) respectively were also obtained following the TLC analysis of the eight paracetamol formulations sampled and the chemical reference substances. Representative chromatogram of the TLC analyses is depicted in Figure 1.

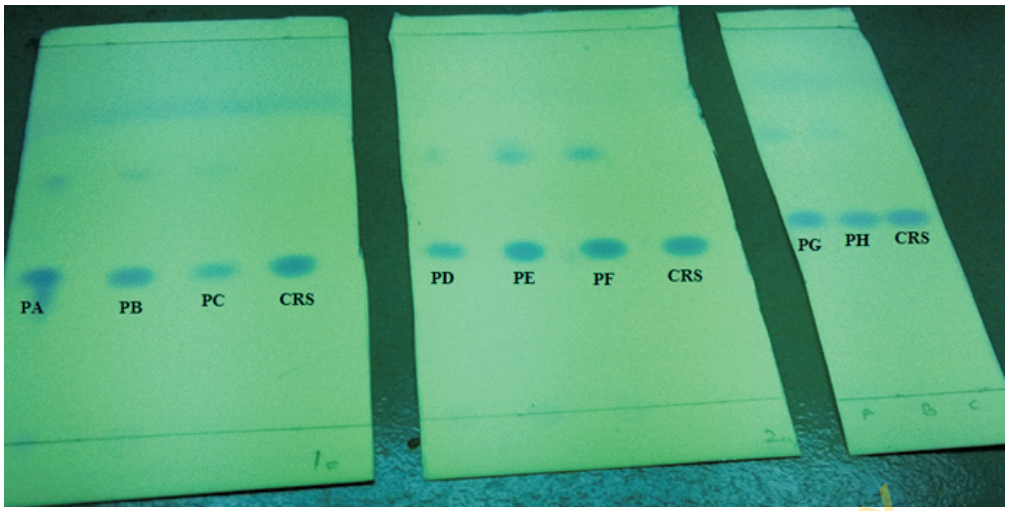

Figure 1: TLC chromatogram of eight paracetamol formulations and reference substance using chloroform:glacial acetic acid:toluene:acetone (13:0.1:2:5) on precoated silica gel plates

Heavy metal analysis

The quantitative estimations of the metals in the five ascorbic acid syrup brands are presented in Table 1. The most abundant metal ions were chromium and nickel which were both present in all the formulations while cadmium was the rarest element detectable in only one of the brands. The product CE consistently showed the highest amount of the metals assayed (except for zinc) and was one of the only two ascorbic acid brands that had detectable levels of lead, the other being CC.

Table 1: Heavy metal content of the various ascorbic acid syrup brands

s/n	Sample	NAFDAC No.	Metal level (mg/L) Cr	Pb	Cu	Cd	Zn	Ni	Co	Mn
1.	CA	Yes	82.48	nd	2.08	nd	3.90	61.8	1.25	0.13
2.	CB	Yes	1.44	nd	0.01	nd	0.97	11.40	0.03	0.08
3.	CC	Yes	5.94	1.27	3.54	nd	65.20	16.80	0.04	0.20
4.	CD	Yes	2.23	nd	0.24	0.02	2.20	14.80	0.03	0.24
5.	CE	Yes	1290.2	0.13	6.30	nd	0.52	1289	2.73	1.07

**nd=not detectable*

The heavy metal contents in the eight brands of paracetamol are depicted in Table 2. The results showed that the most abundant elements in the brands were nickel and chromium in that order which were detectable in all the brands while the rarest ions were those of lead and cadmium which were detectable in PA and PB syrups respectively. In addition, zinc, manganese, copper and cobalt ions were common with all the brands assayed. The PC and PD formulations were the least contaminated with heavy metals amongst the brands tested.

Table 2: Heavy metal content of the various paracetamol syrup brands

s/n	Sample code	NAFDAC No	Metal level (mg/L) Cr	Pb	Cu	Cd	Zn	Ni	Co	Mn
1.	PA	Yes	6.15	0.16	0.28	nd	6.70	13.10	0.03	0.13
2.	PB	Yes	1.16	nd	0.83	0.01	0.53	37.70	0.09	0.38
3.	PC	Yes	1.31	nd	0.03	nd	0.42	2.37	0.02	0.13
4.	PD	Yes	1.46	nd	0.03	nd	0.95	2.61	nd	0.05
5.	PE	Yes	3.93	nd	0.49	nd	5.80	35.00	0.08	0.22
6.	PF	Yes	644.60	nd	2.75	nd	1.59	712.30	1.21	0.73
7.	PG	Yes	2.10	nd	0.07	nd	0.90	11.80	0.04	0.09
8.	PH	Yes	6.13	nd	0.95	nd	0.27	58.00	0.04	0.17

**nd=not detectable*

DISCUSSION

With both paracetamol and ascorbic acid formulations, the extent of elemental contamination varied with the heavy metal (cadmium was present in only two brands, chromium and nickel in all thirteen) and product such as CE showed highest overall contamination including lead. The formulations also contained varying amounts of the trace elements zinc, cobalt, manganese and copper.

Thin layer chromatography using the various mobile phases confirmed the presence of the active ingredients in the thirteen brands of the ascorbic and paracetamol syrups analysed as their chromatogram showed spots identical in R_f values and colour with their respective chemical reference substances. The identity of the active ingredients in the five brands of ascorbic acid was further confirmed with positive basic test results.

The heavy metal analysis showed that nickel and chromium metals were the most common as they were found in all thirteen brands and were also the elements present in the highest concentrations. This might suggest that the source of contamination with the two elements is quite basic and ubiquitous with the drug companies. Nickel and chromium are widely employed in metallurgical processes and in the production of stainless steel tanks with high corrosion and temperature resistance such as those used in the batch manufacturing of liquid formulations. Elemental nickel and its compounds are also employed as catalysts in the organic synthesis of industrial intermediates and as pigments that are widely used for aesthetic enhancement of drugs.[6] In addition, nickel levels as high as 0.75 mg/L in groundwater[23] and chromium concentrations in excess of the WHO permissible limits in drinking water have been reported.[2] Nickel is generally regarded as non-toxic and also essential for biochemical functions, however, sufficiently high levels in consumer products, including food and drugs have been reported to cause allergic dermal reactions.[24] Although elemental nickel is not carcinogenic and only a few nickel compounds including its oxides, sulfides and soluble salts have been shown to increase the occupational risk for developing cancer, the general underlying mechanism of carcinogenicity of these compounds which involve the transport of nickel ions into target cellular organelles necessitated the classification of all nickel compounds as human carcinogens (Group 1) by the IARC[24,25] and is also sufficient concern for regulatory agencies to limit human exposure from consumer products and drug formulations. The International Conference on Harmonisation (ICH) have documented the Permissible Daily Exposure (PDE) of elemental impurities in drug products intended for oral administration.[26] The PDE have been demonstrated to be protective of the toxicological effects of the metals in the general population including paediatric patients. The daily exposure of heavy metals in the eight paediatric paracetamol syrups calculated based on a 15 mL per day dosage for a 20 kg five-year old is depicted in Table 3. The results showed that six out of the eight paracetamol brands contained nickel at levels in excess of the PDE. An additional four of the five ascorbic acid syrup brands also revealed nickel content in excess of the limit set by the ICH as shown in Table 3. Chromium contamination of CE ascorbic acid and PF paracetamol brands was also greater than the PDE. Chromium is better tolerated in humans as its toxicity varies with the oxidation state, ranging from the low toxicity of the elemental chromium to the highly toxic hexavalent form. The ubiquitous Cr (III) is better tolerated in humans compared to the rarer hexavalent form which is a designated carcinogen and which accounts for the majority of chromium toxicity.[2,27]

Table 3: Calculated daily exposure of heavy metals in the thirteen brands

		PDE (µg/Kg/day)**							
	Drug products*	Cr	Pb	Cu	Cd	Zn	Ni	Co	Mn
	PDE	157.14	7.14×10^{-2}	18.57	7.14×10^{-2}	-	8.57	7.14×10^{-1}	-
1	CA	61.86	0.0	1.56	0.0	2.93	46.35	0.94	0.10
2	CB	1.08	0.0	4.5×10^{-3}	0.0	0.73	8.55	0.02	0.06
3	CC	4.46	0.95	2.66	0.0	48.90	12.60	0.03	0.15
4	CD	1.67	0.0	0.18	1.5×10^{-2}	1.65	11.1	0.02	0.18
5	CE	967.50	0.09	4.73	0.0	0.39	966.75	2.05	0.80
6	PA	4.61	0.12	0.21	0.0	5.03	9.83	0.02	9.75×10^{-2}
7	PB	0.87	0.0	0.62	7.5×10^{-3}	0.40	28.28	0.07	0.29
8	PC	0.98	0.0	2.25×10^{-2}	0.0	0.32	1.78	1.5×10^{-2}	9.75×10^{-2}
9	PD	1.09	0.0	2.25×10^{-2}	0.0	0.71	1.96	0.00	3.75×10^{-2}
10	PE	2.95	0.0	0.37	0.0	0.44	26.25	6.0×10^{-2}	0.17
11	PF	483.41	0.0	2.06	0.0	1.19	534.23	0.91	0.54
12	PG	1.57	0.0	0.05	0.0	0.68	8.85	0.03	6.75×10^{-2}
13	PH	4.59	0.0	0.71	0.0	0.20	43.5	0.03	0.13

**C codes = vitamin C, P codes = paracetamol; **Calculated values are based on a 15 mL daily dosage for a 20 kg five-year old.*

PDE-Permissible Daily Exposure of heavy metals in oral drug products. The PDE for zinc and manganese which are classified as low risk to human health are not determined.

The thirteen different brands also contained varying amounts of zinc, cobalt, manganese and copper which although are classified as essential trace elements being required for normal biochemical functions but in excessive amounts can cause cellular damage and diseases in humans. These include epigastric pain, acute renal tubular necrosis and interstitial nephritis with zinc toxicity;[28,29] goiter, reduced thyroid activity, cardiomyopathy, optic and acoustic nerve damage with cobalt toxicity;[30-32] neurotoxicity, cardiovascular toxicity, hepatotoxicity, reproductive and developmental toxicity with manganese toxicity[33, 34] and Wilson's disease in cases of copper toxicity.[35] Unintentional inclusion of these metals into drug formulations could arise from water sources and from their frequent use in metallurgical processes and the manufacture of high-temperature and corrosion-resistant alloys for high-strength steel tanks, their use as industrial catalysts and in pigment manufacture.

Lead was also present at levels above the PDE in PA, CC and CE while cadmium was detectable in PB and CD syrups. These two elements are considered as grave public health concern because of the severity of their adverse effects. Lead has been associated with impaired development in children including low intelligence quotient, delayed neurobehavioural development, poor language recognition and acuity skills and general anti-social behavior.[36-38] The major adverse effect of cadmium is renal impairment.[39] Even when present at concentrations below the permissible daily limits, the toxicity of the metals could be additive or synergistic following human co-exposure to the metals. Heavy metals like lead and cadmium have also been reported to interfere with the normal biochemical functioning of essential trace elements.[2] The effects will be more pronounced in children with immuno-compromised status due to poor nutrition and previous metal exposure from other sources including the environment and diet.[37]

In addition, elemental impurity can contribute to drug instability including the metal ion-catalyzed degradation of pharmaceutical ingredients thereby contributing to shortening of products' shelf lives, aesthetic inelegance and the possibility of adverse effects from degradation products.

Control strategies to limit elemental contamination of drug products include strict compliance to compendial requirements for water, careful equipment and raw material selection, equipment qualification, routine testing and maintenance of equipment and GMP observance. It is also recommended that regulatory agencies include and/or enlarge the scope of heavy

metal analyses of these two products beyond the minimal compendial requirements of the analyses of copper and lead in Vitamin C and lead in paracetamol bulk forms.[20] The present limitation of heavy metal analysis to those listed in Pharmacopoeias will make analysts miss out on the other possible contaminants especially in our third world setting where the water sources are purely built by industries and vessels used for production may be overused. The present study has exposed the need to look out for heavy metals more than those specified in the Pharmacopoeias for paediatric formulations.

A major limitation of this study was that elemental analysis was carried out on brands of only paracetamol and ascorbic acid. Future studies will include other medications.

CONCLUSION

The quantitative estimation of elemental contamination of paracetamol and ascorbic acid syrup brands in the Nigerian market has been carried out and the levels of particular elemental impurities compared with established PDE. The high levels of some of the heavy metals found in the syrups calls for proper implementation of good manufacturing practice.

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