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Determination of Al, Ca, Cl, Cr, K, Mg, Sb and Ti in industrialized and formulated antihypertensive drugs using Neutron Activation Analysis

Josianne N. Silveira[1], Maria A. C. B. Menezes[2], Paulo. C. P. Lara[3], Mark. A. Beinner[4], José Bento B. da Silva[3*]

[1]*Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, Brazil.*

[2]*Nuclear Technology Development Center, Brazilian Commission for Nuclear Energy, CDTN/CNEN, Division for Reactor and Irradiation, Laboratory for Neutron Activation Analysis, Caixa Postal 941, CEP 30123-970, Belo Horizonte, Brazil.*

[3]*Department of Chemistry, Institute of Exact Sciences, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais, CEP 31.270-901, Brazil.*

[4]*School of Nursing and Nutrition, Federal University of Minas Gerais, 30130-100, Belo Horizonte, MG, Brazil*

Abstract: *This work evaluated Al, Ca, Cl, Cr, K, Mg, Sb and Ti levels in capsules and tablets of the formulated and industrialised antihypertensive enalapril maleate and amlodipine besylate drugs used systematically. The neutron activation analysis was applied using the k_0-standardization method. The accuracy was checked with certified materials. The detection limits ranged from 0.01 µg capsule^{-1} for Cr and Sb and 0.01 µg tablet^{-1} for Mg, Sb and Ti, and 400 µg capsule^{-1} to 1000 µg tablet^{-1} for Ca. In the 63 analysed samples, the element levels ranged from 0.064±0.005 µg tablet^{-1} for Sb and 36.2±4.5 mg tablet^{-1} for Ca. In formulated capsules, the levels varied between 0.010±0.001 µg capsule^{-1} for Sb to 30.5±1.1 mg capsule^{-1} for Mg.*

Keywords: *Inorganic constituents, industrialized and formulated antihypertensive drugs, Neutron Activated Analysis*

I. Introduction

Chemical elements are widely distributed throughout the environment, deposited in water, accumulated in soil, in plants and animals through natural processes, like leaching soil or as a product of development of modern technology (atmospheric pollution, anthropogenic sources). In man, besides environmental exposure to chemical elements, by contaminated air, water, foods, he can additionally be exposed to use of conventional and herbal medicines, cosmetics, domestic utensils, increasing the biodisponibility of those metals in the organism [1]. In this review article the author points to studies where contamination was observed primarily with substantial amounts of elements such as Sb (especially in leishmanicidal formulations), selenium in food supplements, as well as As, Cd, Cr, Hg, Ni, Pb and Zn in herbal medicine formulations [1].

Results observed in a study by Flório [2] indicate that medication are most prone to aluminum contamination, principally due to its packaging in exclusively aluminum blister compounds (rather than the traditional aluminum + plastic packaging), mainly for chemical stability reasons and due to their tendency to photolysis, common in antihypertensive and anti-ulcer drugs. According to the author, this phenomenon makes storage of these products a critical factor. Furthermore, and citing an author example, the antihypertensive class of drugs have the greatest presence of contaminants on average demand on the market [2].

According to Oliveira and Scarpa [3], the nature of the containers must be considered both in the production and storage stages of the finished product, since they come into direct contact with the drug. During industrial production, the recommendation is to use stainless steel containers, which are resistant to most substances. Also according to the authors, other metals such as iron, aluminum and copper are incompatible with acidic or basic drug forming complexes often considered to be toxic. Choice of container for final packaging of the product is of fundamental importance for the preservation of product integrity, because in addition to it being the only protective barrier against the external environment, the product is in direct contact with the pharmaceutical formulation [3].

The excipients used in pharmaceutical formulations also contribute to the presence, at times, to high concentrations of other chemical drug elements. In the example of Ca, the Handbook of Pharmaceutical Excipients [4] calls attention to the use of substances which act as carriers of this base metal, such as calcium alginate, calcium carbonate, calcium phosphate, calcium stearate, calcium sulfate, calcium carboximethylcellulose, among others. The excipients for chlorine [4] can include benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, chlorobutanol, chlorocresol, chlorodifluoroethane,

chlorofluorocarbons, chloroxylenol and others. And finally, excipients for K [4] include acesulfame potassium, potassium alginate, potassium benzoate, potassium bicarbonate, potassium chloride, potassium citrate, potassium hydroxide, potassium metabisulfite, potassium sorbate, and others.

In the case of Mg, the principal excipients are aluminum magnesium silicate, magnesium carbonate, magnesium oxide, magnesium silicate, magnesium stearate, magnesium trisilicate.

Most people that suffer from hypertension take medication daily and this leads to the introduction of metals in the organism, which is particularly dangerous to those with a tendency to accumulate metals in their body. Along with other daily possible contamination sources, the metals contained in drugs, when taken daily, can cause several health problems [5].

Hamzah et al [6] studied the ten most popular herbs used in Malaysia by instrumental neutron activation analysis. A total of 16 trace and major elements were determined and the concentration of these elements varied depending on the origin of the herb. The study showed that the toxic elements found in the samples were below the levels stipulated by the Malaysian government health agency. According to mutagenicity tests, there was no observed toxic effect due to heavy metal presence in the studied herbs.

The use of a suitable modifier (ammonium pyrrolidine dithiocarbamate and 8-hydroxyquinoline) in electrothermal vaporization ICP-MS provides a simple technique to determine Cr, Mo, Pd, Cd, Pt, and Pb in antihypertensive tablets without complicated sample preparation. The background ions at the m/z 52 and 53 Cr masses were significantly reduced in intensity by using NH_3 as reaction cell gas in the dynamic reaction cell (DRC). The introduction of dry aerosol with the ETV sampling device and DRC effectively alleviated the spectral interferences. Compared to traditional sample preparation procedures, such as acid digestion and dry ashing, slurry sampling offers several benefits including reduced sample preparation time, reduced possibility of sample contamination, and decreased possibility of analyte loss before analysis; although the benefits of slurry sampling can be affected by the fact that analyte addition calibration must be performed. The values obtained by ETV-ICP-MS were in good agreement with reference values obtained by pneumatic nebulization ICP-MS of completely dissolved samples [7].

Okatch et al [8] determined arsenic, chromium, lead and nickel levels in twelve plant species used in the treatment of opportunistic infections of HIV/AIDS by Flame Atomic Absorption Spectrometry (FAAS) after acid digestion. The dry weight content of these metals was as follows: As (0.19-0.54 µg g^{-1}), Cr (0.15-1.27 µg g^{-1}), Pb (0.12-0.23 µg g^{-1}) and Ni (0.09-0.21 µg g^{-1}). All the determined metals were below the WHO permissive maximum levels. The maximum possible weekly intakes of the heavy metals, following treatment regimes, were insignificant compared to the provisional tolerable weekly intake recommended by WHO [9] and the Joint FAO/WHO Expert Committee on Food Additives [10]. This suggests that heavy metal exposure to patients originating from consumption of traditional medicinal plants is within non health-compromising limits.

Olowoyo et al [11] investigated the uptake and translocation pattern of trace metals from two different plant species from a waste dump site in Pretoria, South Africa. Datura tramonium is used as an anti-asthmatic treatment, and Amaranthus spinosus may either be used as a medicinal herb or consumed as a vegetable. It was concluded from this study that most of the examined elements from the two plants were within the allowable limits, except for Cr in leaves and stems, and Ni in plant roots. The plants collected from this type of waste dump may not pose a serious danger with regards to metal uptake; however, according to the authors, a periodical assessment of plants, used for traditional medicine, should be encouraged, as this will assist in predicting quality assurance and safer herbal use, especially for an urban population where the level of pollution may be very high.

Hypertension is a multifatorial process and the principal cause of illness in industrialized countries [12]. Hypertension, also known as high blood pressure, is defined as systolic blood pressure of 140 mmHg or higher and/or diastolic blood pressure of 90 mmHg or higher. The classification of blood pressure used in the 2007 ESH/ESC Guidelines comprises categories of optimal (systolic blood pressure less than 120 mmHg and diastolic blood pressure less than 80 mmHg), normal (systolic blood pressure 120-129 mmHg and/or diastolic blood pressure 80-84 mmHg), and high-normal (systolic blood pressure 130-139 mmHg and/or diastolic blood pressure 85-89 mmHg) blood pressure, followed by 3 grades of hypertension, and a separate category for isolated systolic hypertension. The 3 grades of hypertension correspond to: 1) mild (systolic blood pressure 140-159 mmHg and/or diastolic blood pressure 90-99 mmHg); 2) moderate (systolic blood pressure 160-179 mmHg and/or diastolic blood pressure 100-109 mmHg); 3) severe hypertension (systolic blood pressure 180 or greater and/or diastolic blood pressure 110 mmHg or greater). Isolated systolic hypertension (systolic blood pressure 140 mmHg or higher), is graded as 1, 2, or 3, according to the systolic blood pressure level, provided that the diastolic blood pressure is less than 90 mmHg. When systolic and diastolic blood pressures fall into different categories, the highest category is used in assessing total cardiovascular risk [12].

The angiotensis-I converting enzyme (ACE) plays an important role in the regulation of blood pressure and hypertension because it catalyses the conversion of inactive angiotensin-I into angiotensin-II, a potent vasoconstrictor and inactivates bradykinin, a potent vasodilator [13]. Consequently, synthetic ACE inhibitors,

such as Captopril and Enalapril, are often used to treat hypertension and other cardio-related diseases. However, these synthetic, ACE inhibitors can cause adverse side effects including cough, taste disturbance, rashes and angioedema [13].

The following work assessed Al, Ca, Cl, Cr, K, Mg, Na, Sb and Ti levels in capsules and tablets in formulated and industrialised antihypertensive enalapril maleate and amlodipine besylate drugs, applying the neutron activation technique, using the k_0–standardization method. The determination of these elements is relevant to the consumer's health and indispensable for quality control.

The neutron activation analysis is an analytical technique for elementary chemical composition determination through induction of artificial radioactivity in a sample, by irradiation with neutrons and subsequent measure of radioactivity [14, 15]. A practical advantage of this technique is that the sample is irradiated without previous chemical preparation, in addition to not having sample losses or contamination from chemical reagents and any alteration of the material chemical composition. Another advantage is the determination of a larger number of elements, not foreseen, with low detection limits.

II. Experimental

2.1. Samples

The samples used in this study were obtained from a local pharmacy for formulated medicines, and some samples were purchased from local drugstores. Two kinds of drugs were selected among the more commonly consumed antihypertensive medications in Belo Horizonte, Minas Gerais, Brazil. The active pharmaceutical ingredients of these two samples included enalapril maleate (EM) and amlodipine besylate (AB). Because local pharmacy stores manipulate different excipients, capsules were manipulated with all of the included excipients, thus these capsules were used as a reference sample (A) for the precision studies described below. The samples included 52 capsules: 42 EM and 10 AB, and 11 tablets: 03 EM and 08 AB.

To check the accuracy of the proposed methodology was used the following certified reference materials: GBW 07604, GSV-3, poplar leaves; GBW 07411, soil; GBW 08501, peach leaves and GBW 09101, human hair distributed by the National Research Center for the Certified Reference Materials in China.

2.2. Application of k_0-instrumental neutron activation analysis

The neutron activation analysis, using the k_0-standardization method [16, 17], was applied to determine the elemental concentration in samples. Irradiation was performed in an IC-7 carrousel of the TRIGA MARK I IPR-R1 reactor at the CDTN (Nuclear Technology Development Center)/CNEN (National Nuclear Energy Commission), at 100 kW, under a thermal neutron flux of 6.35×10^{11} neutrons cm^{-2} s^{-1}. The parameters f and α in the IC-7 are (22.32 ± 0.2) and (-0.0022 ± 0.0002), respectively [16]. The samples were irradiated simultaneously with neutron flux monitor Al-Au (0.1%) IRMM-530RA foil cut into 5 mm diameter and 0.1 mm thick.

The standard neutron activation analysis, including gamma spectroscopy, consisted of the following two protocols: 5 min of irradiation time and suitable decay and measurement time to determine elements with Al, Cl, Mg, and Ti presenting radionuclides short half-lives, and 8 hours to determine elements, which radionuclides present medium – K and Na - and long half-lives – Ca, Cr and Sb. The gamma spectroscopy was performed on an HPGe detector with 15% efficiency, and for the spectra analysis, a peak area evaluation, using the HyperLab program, [18] was used. The software package KAYZERO/SOLCOI [19] was applied to calculate the elemental concentrations.

III. Results And Discussion

3.1. Limits of Detection (LODs)

For determination of the limits of detection (LODs) for each element in the antihypertensive samples, 10 tubes of polyethylene with empty capsules were analysed using the same procedure applied to the above mentioned samples. The tubes were later counted, and using the KAYZERO/SOLCOI program, the detection limits were calculated. Table 1 presents the limits of detection for each analyzed element. The LODs ranged from 0.01 µg $capsule^{-1}$ or $tablet^{-1}$ for Sb, to 400 µg $capsule^{-1}$ and 1000 µg $tablet^{-1}$ for Ca.

3.2. Precision

Precision was evaluated using intra-assay studies. Seven replicates of the reference sample (A) were irradiated separately on the same day and read in triplicate following the protocol described in section 2.2. The intra-assay coefficient of variation (CV) was calculated using the standard deviation obtained for the concentration (µg L^{-1}) for each element in reference sample A and divided by the average and multiplied by 100. Table 2 presents the intra-assay coefficient of variations, which express the precision of the proposed analytical method. The highest intra assay CV, observed in this study, was 14.1 % for Al, and the smallest intra-assay CV was 4.3 % for Na, thus demonstrating good precision. Considering the criteria established by The International

Association of Official Analytical Chemists (AOAC, 1993) [20], CV varied between 15 to 30%, depending on the concentration range (100 μg g^{-1} to 1 μg g^{-1}). The values were within the acceptability range.

3.3. Accuracy

To evaluate accuracy, several certified samples, including poplar leaves, soil samples, peach leaves and human hair [21-23] were irradiated in random rounds following the protocol described in section 2.2. The results of the certified samples represent an average of several determinations conducted during sample analyses. As can be observed in Table 3, the majority of the experimental results are within the range of uncertainty for the certified values.

In spite of the fact that there was no certified reference material similar to the analysed matrix, this data provides support to the concentration accuracy of the results observed in the antihypertensive samples using the neutron activation analysis technique.

3.4. Determination of the sample metal concentrations

Table 4 presents the results of 63 antihypertensive samples analysed by neutron activation analysis. Essential macro elements were among the elements found in the analysed samples Na, K, Mg, Cl and Ca. Chromium was an essential ultra-trace element, and Al, Sb and Ti were environmental micro-contaminants. Underwood [21] defines 26 elements as essential to a healthy human being. The essential elements C, H, N, O, P, Ca, S, Cl, K, Mg and Na are typically expressed in the grams/100g range, and the essential elements Fe, I, Zn, Se, Mn, Cu, Cr, Mo, Co, Ni, F, Sn, SI, V and As are expressed as mg g^{-1} and ng g^{-1}. These 26 elements are related to human health, in addition to diseases, once their deficiency or excess can induce physiologic changes in the body [22]. Trace-elements are distributed into 3 groups according to the World Health Organization (WHO) [23], in function of their nutritional significance in humans: essential trace elements are I, Zn, Se, Cu, Mo, Cr, Fe and Co, and probable essential trace elements include Mn, Si, Ni, B, and V. A third group of trace elements, which can be potentially dangerous, however, present some essential functions at low concentration levels and include F, Pb, Cd, Hg, As, Al, Li and Sn. In accordance with the WHO [23] recommendations and the National Research Council, expressed as the Recommended Dietary Allowances (RDA) [24], daily ingestion of trace elements is as follows: for Ca, the RDA is 320-480 mg, and for WHO, daily ingestion is 160-200 mg; for Cl, RDA is 300 mg; for Cr, the RDA is 80 μg; for K, RDA is 800 mg; for Mg, RDA is 112-140 mg, and for WHO: 120 mg; for Na, the RDA is 200 mg, and for Sb, the recommendation in adults is 20 μg, when considering a 40% percentile for a meal. Table 4 shows that only 4 samples contained Na. Fifty-nine samples presented a Na concentration that ranged from 2.8 μg $capsule^{-1}$ to 6.0 mg $capsule^{-1}$. This difference can be explained by the use of sodium bicarbonate or sodium lauryl sulfate as an excipient in the same formulation for capsules or tablets. Sodium concentrations, expressed in milligrams, should be carefully observed by the physician and the hypertensive patient. The Na concentration intake in a sodium restricted, patient's diet, which is approximately 2400 mg day^{-1}, should be controlled in order to avoid health complications. The trace element Ti was only observed in formulated capsules, which may be due to titanium's link to pigments used in capsule production by the pharmaceutical industry.

The lesser of the trace elements observed in these samples were Ca, Cr, K and Sb. Of the 63 analyzed samples, four capsules presented Cr concentration higher than the recommended daily ingestion (80 μg), which may be explained by contamination from stainless steel utensils used in capsule preparation. Chromium absorption is variable in function of the type of ingested composition [25]. The insoluble compositions of Cr (III), as chrome oxide, are virtually non-absorbed in the gastrointestinal tract; 0.5 to 2% of the composed Cr (III) presented in the diet is absorbed, and approximately 2 to 10% of Cr (VI), as potassium or sodium chromate, is absorbed in the tract [26]. The half-life of Cr elimination varies from 35 hours to 4 days, depending on the type of composed chromium that is absorbed [27]. Chromium is a micro essential element, however, its toxicity depends on its oxidation state, given Cr (VI) possesses higher toxicity than Cr (III), a speculation for its greater ability in transposing membranes [28]. Antimony (Sb) is not an essential element but it is potentially toxic. It is well established that metalloid solution concentrations can increase with increases in pH in an acidic system, and toxicity can also increase as the system is reduced. These changes in mobility and toxicity depend on the Sb associations with other components present in the matrix [29]. However, its concentration in samples is in the order of the ng $capsule^{-1}$ or $tablet^{-1}$.

Magnesium (Mg) is an essential element in biological systems, and in sample concentrations, it was found to be elevated, in the range of 1 to 30 mg $capsule^{-1}$, which would correspond to the 25% highest value of the recommended level of daily ingestion. This can be explained by the use of magnesium stearate as an excipient used by manufacturers [4]. The presence of Al in the 27 samples is thought to come from contaminated air, raw material, utensils, and or industrial machinery. It should be emphasised that aluminium is not an essential element for biological systems [30]. The analysed levels in the medication are not worrisome since oral absorption is low, ranging from 0.1 to 0.5% of the ingested dose (average of 20.5 mg), with rapid

elimination (between $t_{1/2}$ 8 hours and 9 days, depending on the exposure) [30, 31]. The presence of aluminum in the thin intestine can result in physiologic consequences, such as the competition with iron, fluoride, strontium, and calcium present in this organ, which results in the formation of complex, insoluble and, consequently, a decrease in absorption of these inorganic elements [30]. Furthermore, this element can accumulate in the body resulting in further complications for individuals with chronic renal insufficiently.

TABLES

IV. Conclusion

From this work, we conclude that the neutron activation technique applying the k_0-standardization method was a suitable technique for determination of Al, Ca, Cl, Cr, K, Mg, Na, Sb and Ti in capsules and tablets of the most commonly consumed antihypertensive drugs in Belo Horizonte, specifically, enalapril maleate and amlodipine besylate. The accuracy was checked with certified materials. The highest values observed for each element in capsules were 129 μg capsule^{-1} for Al, 18495 μg capsule^{-1} for Ca, 598 μg capsule^{-1} for Cl, 1864 μg capsule^{-1} for Cr, 3761 μg capsule^{-1} for K, 30537 μg capsule^{-1} for Mg, 6030 μg capsule^{-1} for Na, 0.4 μg capsule^{-1} for Sb and 1686 μg capsule^{-1} for Ti. In analysing the tablets, the highest values were 47 μg tablet^{-1} for Al, 27566 for Ca, 588 for Cl, 1.8 for Cr, 1216 for Mg, 1461 for Na and 0.12 for Sb. Potassium and Ti were not detected in tablets. In spite of concentration levels of certain elements in antihypertensive medication being below the recommended ingested values, with the exception of Cr, it should be emphasized that these observed contaminant values in drugs will be added to the values found in food, as well as water, and other sources of contamination for each individual. The concentrations of Na were below the acceptable daily ingestion value; however the analysed medications are used by hypertension individuals that follow a restricted diet. Doctors and patients, unaware that Na exists in capsules or in tablets, will fail to include this value when summing the values of Na in a restricted-sodium diet. This information is important for local pharmacies, drugstores and the pharmaceutical industries, for they can substitute the excipients that contain Na, as sodium bicarbonate to sodium lauryl sulfate. On the other hand, because of inherent toxicity of each metal, their determination in medication is necessary to avoid larger damages to patients.

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