IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 3 Ver. III (May - Jun. 2015), PP 62-72
www.iosrjournals.org

Determination of lead in herbal syrups by graphite furnace atomic absorption spectrometry after multivariate optimization

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Abstract: *In this work, a method to determine lead in herbal syrups by graphite furnace atomic absorption spectrometry (GF AAS), after simple and rapid preparation of samples (with syrup sample 20% v/v, nitric acid 1.2% v/v, Triton X-100 1.2% v/v and water to complete the volume for 1.0 mL) and multivariate optimization of the main variables of interest, is presented. The best modifier was permanent tantalum (520 µg) on the L'vov platform with pyrolysis and atomization temperatures of 700and 1900ºC and pyrolysis time of 50s. The calibration was accomplished with aqueous standardsolutions (external calibration) with a working calibration range of 0.0 – 28.0 µg* L^{-1}*. The limits of detection and quantification were 1.6 and 5.5 µg* L^{-1}*, respectively, with an average characteristic mass (n=5) of (5.3 ± 0.2) pg (recommended 5.0 pg). A spiked sample recovery (in four concentration levels of Pb) was between 99.6 and 106.7%. The precision intra- and inter-assay (in four concentration levels of Pb) was between 5.3 and 5.9% and 4.0 and 13.9%, respectively. The lead concentrations obtained in six different syrups were between 6.6 to 15.9 µg* L^{-1}*.*

Keywords: *GF AAS, Lead, Herbal Syrups, Multivariate Optimization, Permanent Modifier*

I. Introduction

The treatment of human disease, using plants as herbal medicine, is as old as human history itself. The discoveries of the application of herbal medicine resulted entirely from empirical or intuitive methods [1].In Western civilization, however, the use of plants as medicine, until recently, was restricted to a specific population group. This use has expanded considerably in recent years, a phenomenon that is mainly due to the elevated cost of drugs manufactured by large pharmaceutical industries, an increasingly difficultreturn on medical and pharmaceutical quality care, in addition to increased consumer confidence and acceptance of natural products [2-4].

According to Tomazzoni*et al*. [5] an increase consumption of herbal medicines may also be associated with a consumer's ability to question the dangers of irrational use of allopathic medicines. Such is the case that evidence basedherbal therapeutic action has been associated with a consumer's dissatisfaction with their health care providers,thus motivating them to seek outalternative medicine[5].

Although herbal medicines are currently used by approximately 80% of the world population, critics warn of their risks, because in many products there is little supporting evidence of their safety and efficacy [6].Although herbal medicines are often times promoted as natural and thus harmless, in no way are they free from adverse effects [4].Several medicinal herbs such as ginsing, aristolochic acid (banned in the US),dietary supplements (nutraceuticals) and megavitamins and their mixtures may present a health risk due to the presence of toxic elements likePb, Cd, Al, Hg and other elements [7-10].

Use of the word quality is defined as a set of criteria characterizing both raw material and its end product. Efficiency is defined by stringent testing evolving preclinical and clinical pharmacological trials for observingbiological effects. Medication safety is determined by testing to demonstrate the absence of toxic effects in addition to the presence of unhealthy contaminants, which may include heavy metals, pesticides, microorganisms and their by-products [11].

In 2000, the Brazilian version of the US Food and Drug Administration, under Resolution No. 17, established quality control standards for herbal medicines as a prerequisite to product registration, which must also includes an analysis of heavy metals, this according to the pharmacopoeial criteria [12].

The toxicity of various elements has been well known and well documented for many years. Early in the 2nd century BC, it was evident that exposure to lead (Pb) could be harmful to human health. Toxicologists continue to study the effect of elements on humans, in addition to periodically reviewing the extent ofpermitted

exposure limits of these elements according to safety data, resulting in the need to perform further pharmaceutical testing for purity for manyof these elements such as Pb[13].

The test for heavy metals in United States Pharmacopeia (USP - 231) and similar testing, such as described in the European Pharmacopoeia (European Pharmacopoeia - EP), the Brazilian Pharmacopoeia (Brazilian Pharmacopoeia 5th Edition) [14] and the British Pharmacopoeia (British Pharmacopoeia - BP), consists of the formation of solid particles of heavy metal sulfides in suspension, and subsequent visual comparison of the color intensity in the sample and standard preparations of a lead solution in a Nessler tube.According to this particular test, there is no identification ofthe elements responsible for a positive result, and furthermore, the testis nonspecific, results in low sensitivity, istime consuming and possesses a low recovery [14-16].

The USP ensures that metals that normally respond to these essays are Pb, Hg, Bi, As, Sb, Sn, Cd, Ag, Cu and Mo, making nodistinction [17].

Heavy metals, such as lead and cadmium in pharmaceutical products, pose serious health risks, even at very low doses [18, 19]

Constant lead exposure can cause adverse effects on psychological and behavioral activities in humans. Blood lead levels in children, once thought to be harmless (below 10 mg / dL), have now been associated with adverse neurocognitive effects [20, 21].A Pb intake of 0.06 mg per day for a period of one month is sufficient to cause chronic poisoning. This level of toxicity causes chronic renal dysfunction, osteomalacia and obstructive pulmonary diseases [18]. These observations warrant continued vigilance to identify lead exposure sources and reduce them where possible [22].

As a heavy metal, lead is widely used in industry, and when ingested or inhaled, it is highly toxic to humans irrespective of age. People can be exposed to lead through lead-containing soil, dust, water, food, air, cosmetics, ceramics, and, notably, the use of drugs contaminated with this metal. Compared to adults, children are more vulnerable to the risks of lead exposure and adverse effects as a result ofrepeated exposure, mainly due to their physical and behavioral characteristics [23].

Low blood Pb levels of roughly 100 mg L^{-1} (as defined by the Centers for Disease Control and Prevention - CDC), lead poisoning in children may also result in growth retardation and cognitive development, in shorter attention spans and behavioral problems [24-26].

A research study made by Hina*et al*. [27] suggests that heavy metals are a major cause of widespread occurrences of respiratory tract infections. A variety of medication, that usevegetable and/or plant extracts as their principle active ingredients, are known for their potentialto treat and/or manage infectious and chronic conditions in different respiratory tract infections (cough, bronchitis, allergies, asthma, nasal congestion and chest pain). As these plants can be natural sources of toxic metals, metalcontamination must not be ignored in herbal medicines.

This study aimed to employ optimization chemometric tools (factorial design and central composite design), in syrup samples prepared by simple dilution (five times) with a mixture of Triton X-100 and nitric acid for the determination of lead in herbal syrups with acceptable accuracy and precision by GF AAS. Once the method was validated, six samples of children's herbal syrup, sold in pharmacies in the city of Belo Horizonte, Minas Gerais, Brazil, were analyzed.

II. Materials and methods

Equipments

In this study, we used a Zeeman 220 (Victoria, Australia) graphite furnace atomic absorption spectrometry (GF AAS) connected to a polarized Zeeman background corrector and an autosampler from Varian®, model SpectrAA. A hollow cathode lead lamp from IST (Imaging & Sensing Technology, Horseheads, NY,WL22927, serial No. 32429) was used, operating at a 7 mA with a 0.5 nm slit widthand 283.3 nm wavelength. Argon N50 (99.999%) from Air Products® (Belo Horizonte, MG, Brazil) was used as a purge gas at a flow rate of 3 L min^{-1}. Graphite tubes (part number 63-100023-00) and L'vov platforms (part number 63-100024-00) were employed for all experiments.

Reagents

All solutions were prepared using deionized water, purified prior to use with a specific resistivity of 18.2 mΩcm^{-1} obtained by the water treatment system from Millipore (Bedford, MA, USA). 65% Suprapur® nitric acid (HNO_3) (Merck, Darmstadt, Germany, Part No. 1.00441.0250) was used in the solutionpreparations and as a washing agent. The standard calibration solutions were prepared starting from the Pb stock solution (1.000 ± 0.002) mg L^{-1} (Titrisol - Merck, part no. 1.09969) in 5% v/v HNO_3. Additionally, we used the followinganalytic solutions (1.000 mg L^{-1}): palladium (Fluka, Buchs, Switzerland) in HNO_3 (1 mol L^{-1}); iridium, ruthenium, rhodium, niobium and tantalum (Fluka, Buchs, Switzerland) prepared in HCl (1 mol L^{-1}) and

tungsten, prepared by dissolving 0.18 g of Na_2WO_4 (Merck, Darmstadt, Germany) in 100 mL of deionized water (Milli-Q).

The polypropylene volumetric flasks, automaticautosampler vials, plastic tubes, micropipette tips and general glassware were left to soak in HNO_3 20% (v/v) for at least 24 hours and rinsed several times with deionized water andair-dried inside a verticallaminar flow hood (VECO, series FL-5998, Campinas, São Paulo, Brazil). As was the case with washing the automatic autosampler, a 0.1% (v/v) Triton X-100 solution was used (Merck, part number 1086431000) in a 0.2% (v/v) suprapurnitric acid solution (Merck) to clean and prevent adsorption of analytes on the flask surface and clogging of the capillary autosampler, in addition to avoidingsample solution dispersion from the platform. The experiments were performed in accordance with the conditions specified and presented Table 1.

According to the manufacturer, when 10 µL of a standard solution of 27 µg L^{-1} of lead is injected in HNO_3 2% (v / v) in the graphite furnace, absorption is about 0.200.This specification was tested to evaluate response, that is to say, the equipment sensitivity.

Sample

For the optimization of the present method, we used a mixture of four different syrups for use by children, composed of honey, propolis and plant extract.

By mixing these syrups to a 1:1:1:1 proportion, the reference sample was obtained and used to standardize and validate the proposed method.

In addition to the four mentioned syrups, two were subsequently acquiredand analyzed, separately, after validation of the method for the determination of Pb.Theses syrups,which are frequently used for the treatment ofcoughing in children and adults, were purchased at local pharmacies in Belo Horizonte-MG (Brazil).

Preparation of the analyzed solutions

Thestandard solutions used for standardizing the use and validation of the developed methodology were prepared as follows: a reference sample of 20% v / v; nitric acid at 1.2% v / v, Triton X-100 at 1.2% v / v; lead analytical solution (in varying concentrations) and deionized water to complete the 1.0 mL volume.

A variable amount of standard lead solution was added to the mixture, and the univariate and multivariate optimization solutions contained 27 µg L^{-1}. During the parameters of merit studies, the added amount corresponded to the concentrations set for the calibration curve and for studies of precision and accuracy.

Univariate optimization

Starting from the conditions of time and temperature, as recommended by the manufacturer (Table 2) and using a standard Pb solution of 27 µg L^{-1} in the matrix (reference sample), absorbance readings were performed by adjusting the conditions of temperatures and times corresponding to the drying, pyrolysis and atomization stages, using a graphite tube with L'vov platform and without chemical modifier. This initial study was required since the matrix syrup is complex and analytic conditions recommended by the manufacturer (for the determination in water) were not suitable for the analysis in question.

Readings were also carried out using different permanent chemical modifiers, treating L'vovplatforms with 520 µg of iridium, rhodium, ruthenium, niobium, tantalum or tungsten [28, 29] using the temperature program proposed by the manufacturer's, as well as the absence of modifier and use of chemical modifier solution (5 µL palladium) in order to facilitate the removal of the organic matrix, thereby liberatingatomization in the analyte.

Once the temperatures and times of pyrolysis and atomization were established, under minimumconditions to obtain an analytical signal, we selected the two best permanent chemical modifiers, and only thendid we proceed with further implementation of the 2^{4-1} factorial design.

Multivariate optimization

Using the 2^{4-1} factorial design, simultaneously, the variables temperature and pyrolysis time, atomization temperature and permanent chemical modifier were tested. This experiment is presented in Table 3.The statistical analysis of the results was performed using the software Statistica 6.0 for windows[30].

Parameters of merit

The investigated parameters of merit in this study were linearity, applying linear regression by ordinary least squares method, resulting ina straight line equation and the determination coefficient (R^2). To evaluate this parameter, the linearity curves in water and the matrix were constructed, in triplicates, using 0, 3, 8, 13, 18, 23 and 28 µg L^{-1}lead concentrations. We also evaluated the matrix effect, specifically, a more, elevated complexity

and viscosity of the syrup matrix, even after diluting five times during the preparation process. The matrix effect study was performed taking advantage of the linearity data constructing triplicate calibration curves with and without the syrup matrix, using a 0 to 28µg L^{-1}Pb concentration range.

The application of the F- and Student's t-tests allowed for a statistical comparison of the slopes and intercepts obtained from the linear regression curves. Next, we determined the limits of detection (LOD) and the limit of quantification (LOQ). To determine the LOD, ten blank solutions and a calibration curve to calculate the concentration were prepared. The equation LOD was calculated as LOD = 3 x S_{Br}/b, in which S_{Br} is the standard deviation of the 10 measurements of different blank solutions, read in triplicate, and b is the slope of the calibration curve. The same procedure, described above, was employed to determine the limit of quantification. The LOQ was calculated as the equationLOQ = 10 x S_{Br}/b[31].We also evaluated the obtained characteristic mass. The characteristic mass is the analyte concentration corresponding to a 1% absorption (or absorbance of 0.0044) and was calculated using data from a calibration curve for each concentration of the analyzed standard solutions using the equation:

$$m_o(pg) = \frac{\text{Standard Pb concentration (µg/L) x standard injected volume (µL) x 0.0044}}{\text{(Analytical solution absorbance of injected Pb - Blank absorbance)}}$$

The accuracy (and the precision)of the method was also evaluated by intra and inter-assay studies. The intra-assay precision was evaluated from the absorbance reading of the reference samples added to 1.5; 3; 5 and 15 µg L^{-1} of the standard Pb solution, prepared in setuplicate and read in triplicate on the same day by the same analyst. From the calibration curve, their concentrations were determined and the intra-assay variation coefficient was calculated. The inter-assay precision was evaluated by repeating the same procedure as above on three consecutive days. The data corresponding to day01 of precision were also used for recovery calculations (n = 7 replicates for each distinct Pb level).

Analytical application

After validation of the method, six samples of herbal syrups were analyzed following the validated method for determining the lead concentration. Samples were prepared in duplicateand diluted 1:5 in a solution of Triton X-100 1.2% and HNO_3 1.2% (prepared as described in the section on analyzed solutions) solution. To calculate the concentration, a calibration curve was prepared by adjusting the concentration range of standard lead from 0 to 7.5 µg L^{-1}, once the samples were diluted 1:5.

III. Results and discussion

Univariate optimization

The validation methodology involved a minimum sample preparation, the addition of nitric acid acting as an oxidant, in addition to preventingmetal solution transfer into the container. Triton X-100, a detergent solution, was also used, aiding in the sample release, which is also capable of reducing carbonaceous deposition residues on the L'vov platform. The reference sample was useful for optimizing analytical conditions, because in the example of using such a diverse sample, one would expect that the standard condition would respond to any other syrup type.

According to the analytical conditions, as established by the manufacturer (Table 2), a 27 µg L^{-1} solution of lead in water should provide an absorbance of approximately 0.200 for a 10 µL injected volume. When testing this specification, nonetheless, using the reference sample (1:1:1:1 syrups mixture), the observed absorbance was 0.1107, which demonstrates that equipment response was less than expected. However, this fact did not negatively contribute tothe developedmethod, as was expected, since the optimum analytical conditions had not yet been achieved.

The graphite furnace temperature and time conditions, as recommended by the manufacturer and described in Table 2, apply to water matrix solutions. To adapt these conditions to the complex syrup matrix, it was necessary, in large part, to modify the recommended program times and temperatures. Therefore, it was convenient to begin the optimization of analytical conditions using a univariate analysis in such a manner that an analytical signal could be obtained while screening for the best results, and consequently, the best conditions for future improvements.

First, temperatures and times were altered relative to the drying stage, while maintaining the other stages constant. Some stages are added between those described in Table 2 (stages 1, 2 and 3) so that drying would be effective without sputtering, analyte loss and absence of excessivebaseline noise.

For pre-selection of the pyrolysis temperature, atomization and permanent chemical modifier, we analyzed the analytical signal profile and the relationship between absorbance and background for each reading. During pyrolysis, the temperature should be one that simultaneously provides the lowest background absorbance correction possible (this shows that there was no formation of carbonaceous residues).As for the atomization

temperature, it should be the lowest possible temperature in which to provide the maximum absorbance for achieving greater sensitivity without compromising thelife and performance of the graphite tube. Thus, the greater the ratio between the absorbance and the background, the better one would expect the employed conditions.

As Table 4 shows, ruthenium, tantalum and iridium presented the best results (signal/background ratio) in the selection of chemical modifiers. Moreover, the absorption backgrounds, containing these permanent modifiers, were below 0.120, after being corrected by Zeeman. To preventcarbonaceous residuesformations and,thus be able to obtain the observed levels of background absorption, a Triton X 100 solution (0.2% v/v) was pre-injected into the graphite furnace. Additionally, we also observed that the sensitivity obtained with ruthenium was well below the other two. Thus, tantalum and iridium were selected to compose the factorial optimization program of multivariate analysis.

Multivariate optimization

Through this experimental design, the results for the univariate optimization were greatly refined: the best pyrolysis temperature was 700 °C and the best modifier was tantalum. The Pareto chart (Figure 1) shows that only these variables, and the relationship between them, were statistically significant at a 95% confidence interval. Since only one quantitative and another qualitative variable were statistically significant, it was not possible to perform the central composite design, which uses at least two quantitative variables.

Thus, either one of the two analyzed values for the other variables-- pyrolysis time and atomization temperature- could be chosen. The pyrolysis time of 50s, 10s more than the tested (40s) was selected to ensure a better matrix destruction so that calibration could be carried out with aqueous standards (no matrix effect), as well as a lower background value, whereas for the atomization temperature, the lower value was selected, 1900 °C, to minimize reduction of the graphite tube performance life.

Table 5 presents the graphite furnace time and temperature program after completing the optimization of the analytical variables.

Parameters of merit

The results obtained for the merit parameters (linearity, linear working range, characteristic mass, detection and quantification limit) are presented in Table 6. The selected method was evaluated by studying the effect of applying the proposed matrix of three aqueous calibration curves (external) in addition to the three curves obtained by matrix-matching calibration method. The slopes of the straight lines, obtained by linear regression, are presented in Figure 2. The F-test indicated that the slope and intercept variances were statistically equal. By applying the T-test, we observed that calculated *t* was less than tabulated *t* ($p < 0.05$). Thus, we conclude that there is no matrix range effect in the utilized concentration, and therefore, aqueous calibration is possible. Furthermore, we observed a visual tendency divergence for the two curves with increasing concentration.This could possibly indicate that the greater concentrations than the range studied here would imply a matrix effect. And in this case, the curve would be by matrix adjusting (matrix-matching calibration).

According to the data presented in Table 7, the observed method demonstrated good accuracy (99.6% to 106.7%), as the recovery values for the four levels are within the acceptable range of 80-120% [32].

The accuracy results, evaluated as the repeatability and reproducibility part of the method, are presented in Tables 8 and 9. It was observed that the coefficients of variation determined for intra-assay precision were<6% and <14% for inter-assay precision, both within the acceptable range: 30% for concentrations equal to 1 ppb and up to 21% for 20 ppb concentrations, this, according to the International Association of Official Analytical Chemists[33]. The limits of detection, quantification and characteristic mass were 1.6 µg L^{-1}, 5.5µg L^{-1} and (5.3 ± 0.2)pg, respectively. The limit of detection and quantification, calculated as described in the experimental section, were above the expected values for the concentration, once precision and recovery of intra- and inter-assay for lead concentrations 1.5, 3 and 5 µg L^{-1} are suitable as mentioned above (Tables 7, 8 and 9). Overestimated concentration values can be justified bythe presence of Pb in the matrix reference. The characteristic mass, as recommended by manufacturer, is a 5.5 pg lead aqueous solution.

Hina*et al*. [27]observed a maximum measured concentration of lead in a sample of 277.44 µg day^{-1}, while the minimum concentration was 0.86 µg day^{-1}.

In an earlier study, Sales and colleagues[34] presented a paper comparing different methodologies for collecting propolis in relation to their lead levels, determining the metal by ET AAS and UV-VIS. In the determination of lead by ET AAS, the authors obtained optimum temperature for pyrolysis and atomization of 850 and 1800 °C, respectively, using a 0.06 mg of magnesium nitrate as a chemical modifier, and using the calibration technique of adding the analyte. By adding 5, 10, 15 and 20 µg L^{-1} of lead, the authors obtained a mean metal recovery of 95.75%, with only one sample done over a single day.

Elsewhere,Orisakwe andNduka [19] analyzed lead and cadmium levels in manufactured pediatric syrups commonly administered in Nigeria. Fifty different syrups samples were incinerated and subsequently

digested using aqua regia (HCl: HNO_3 - 3:1) and their lead and cadmium contents were analyzed by FAAS. The detection limit obtained for lead was 10 µg L^{-1}. Interestingly, in this study, the authors did not present data on the accuracy or precision in their methodology.

In a recent Nigerian study, Nkeiruka *et al*.[35]presented a paper on the content of heavy metals (As, Cd, Cr, Co, Pb and Zn) in herbal medicine comparing the observed results with the proposed limits by the World Health Organization (WHO), European Union (EU limits) and United States Environmental Protection Agency (US EPA). To this, 22 herbal remedies were used in two forms: liquid and capsules were digested with aqua regia andtheir metal contents were determined by FAAS. The detection limit for lead was 5µg L^{-1}. In their work, the authors do not present nor cite data on the accuracy of the methodology or the obtained accuracy.

Ting*et al*. [36] presented a paper and discussed microbial and heavy metals contamination (Pb, Mn, Cu, Cd, Fe and Zn) in medicinal herbs traditionally consumed in Malaysia, and on the effect of cooking before ingestion in relation to the levels of contamination. To this end, eight kinds of traditional Chinese herbs were purchased in local shops. For metal dosages, in each 10 g of sample, 200 mL of distilled water (performed in triplicate) was added in 500 mL of Schott Type vials. Then the flasks were shaken on a rotary shaker for 1h at room temperature (27 ± 3) °C allowing for good sample mixtures. After filtering the solutions, the levels of Mn, Cu, Cd, Pb, Fe and Zn were analyzed by FAAS. For the post cooking studies, herbs were boiled at 100 °C for 1 h and analyzed by the same procedure. Unfortunately, in this study, there was alack of discussion as to theaccuracy or precision methods, nor were there any results showing the limits of detection and quantification.

In yet another, earlier study, using ET AAS, Lima*et al*. [37] presented a methodology for direct determination of lead in energy drinks with fruit flavors, syrups and honey. The authors began by weighing 4 grams of each sample, which were then completely dissolved in water and acidified with HNO_3 at 0.2% v / v, resulting in a final volume of 50.0 mL.After that, volumes of 20 µL of each sample, plus 10 µL of a chemical modifier mixture [Pd $(NO_3)_2$ 0.05% m/v + Mg $(NO_3)_2$ 0.03% m/v], were directly introduced into a graphite furnace with transverse heating (THGA). Following this procedure, Pb recoveries were observed between 90.2 to 106%, with a mean of 97.1%, good repeatability (RSD <6%) and with a characteristic mass of (15 ± 1) pg.

Analytical application

Oncethe method validation was concluded, we determined the lead concentrations in samples as presented in Table 10. The observed lead concentration result in the six (1 to 6) analyzed samples was (6.6 ± 2.5) µg L^{-1} to (15.9 ± 2.5) µg L^{-1}. Results such as these in the studied herbal syrups are not surprising, since the syrup amount administered daily to children was no greater than 100mL.Nevertheless, as a general rule, Pb absorption in the gastrointestinal tract of children is roughly 50%[38].

In a study by Sales *et al*. [34] the authors observed a lead content ranging from 7.0 and 8.9 mg kg^{-1} propolis collected using wedges, while propolis collected by a meshing method presented results ranging from 1.2 to 1.8 mg kg^{-1}. These observed lead levels were lower than the maximum limit set by the Japan Propolis Conference (20 mg kg^{-1}) and, in the case of the mesh method, were inferior to the limit set by Codex Alimentarius for foods in general (2 mg kg^{-1}) .

In a study by Orisakwe and Nduka[19]which studied lead and cadmium levels in syrups by FAAS, the authors reported lead levels ranging between undetectable (less than the detection limit of 10 µg L^{-1}) to 1.08 µg L^{-1}. In another study [35],in which the authors analyzed herbal medicines marketed in Nigeria, the lead levels ranged from 0.57 to 20.6 µg L^{-1}.

In a studymade by Ting*et al*.[36] using herbal medicines, for all the metals studied (except for Mn, which had the highest level of 18.5 mg L^{-1}) concentrations of lead were below 1 mg L^{-1}. It is also worth noting that boiling herbal medicines (at 100 °C for 1 h) was only effective in reducing copper, while having little effect in altering the levels of the other studied metals.

In work done by Kauffaman*et al*. [22] which analyzed lead levels in pharmaceuticals and dietary supplements, levels ranged from 0.05 µg L^{-1} (in an infant diphenhydramine oral solution) to 500 µg L^{-1} in multivitamin chewable tablets with fluorine and iron.

In another study by Obi *et al*. [39]with herbal medicines consumed in Nigeria, the authors observed that the daily intake of lead from these herbal drugs may be greater than 514 mg per day. And finally, in work done by Lima*et al*.[37]analyzing two syrups in beverages, the authors reportedlead levels between 29.3 to 45.4 ng g^{-1}.

IV. Conclusion

The developed method permitted the determination of lead in herbal syrups by graphite furnace atomic absorption spectrometry (GF AAS), using a simple and fast sample preparation. Digestion was carried out effectively during the pyrolysis stage in a graphite tube with an L'vov platform treated with 520 µg of tantalum, allowing for a relatively high Pb temperature and matrix elimination, which can be verified by the absence of matrix effect and the possibility of the calibration method using aqueous solutions (external calibration).

Nevertheless, the optimized furnace program and the use of tantalum as a permanent modifier provided for adequate sensitivity and background correction for the complex syrup matrix.

Multivariate optimization for the standardization of analytical conditions permitted use of a small number of experiments, thus reducing analysis time and cost. Validation demonstrated that the method for lead determination is precise and accurate, and that it is possible to use aqueous calibration in the lead range of 0 to 28 μg L^{-1}.

Given the determined lead sample concentrations, we observed that, indeed, there exists Pb contamination of syrups, although not at what would be considered alarming levels (less than 16 μg L^{-1}).

This study achieved its objectives, which implies future usefulness for the quality control of herbal syrups, and set precedents for initiatives to develop methods to address the contamination by other metals in this class of matrices, increasingly used as an alternative to allopathic medicines.

Acknowledgements

The authors wish to thank the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for their financial support.M. S. Dias is greatful to FAPEMIG for the scientific scholarship. J. B. B. Silva isthankfultothe Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for thescientificresearchscholarship.

References

[1]. E.D. Caldas, L.L. Machado, Cadmium, mercury and lead in medicinal plants in Brazil. Food and Chem. Toxicol.,*42*, 2004, 599-603.
[2]. A.D. Corrêa, R.S. Batista,Plantas Medicinais, do cultivo à terapêutica, Ed. Vozes, pp. 15-110,1998.
[3]. L.L. Machado, Dissertação: Cádmio, chumbo e mercúrio em medicamentos fitoterápicos. Mestrado em Ciências da Saúde Faculdade de Ciências da Saúde, Universidade de Brasília,2001.
[4]. S. Basgel, S.B. Erdemoglu, Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. Sci. of the Total Environ. *359*, 2006, 82-89.
[5]. M.I.Tomazzoni, R.R.B.Negrelle, M.L. Centa, Fitoterapia popular: a busca instrumental enquanto prática terapêutica. Texto & Contexto Enferm. *15*, 2006, 15-21.
[6]. P.W. Woods, Herbal healing. Essence30, 42-46,1999.
[7]. E. Garcia, C. Cabrera, M.L. Lorenzo, M.C. López, Chromium levels in spices and aromatic herbs. Sci. Total Environ. *247*, 2000, 51-56.
[8]. N.Lekouch, A. Sedki, A. Nejmeddine, S.Gamon, Lead and traditional Moroccan pharmacopoeia. Sci. Total Environ. **280**,2001, 39-43.
[9]. F.F. López, C. Cabrera, M.L. Lorenzo, M.C. López, Aluminium levels in spices and aromatic herbs. Sci. Total Environ. *257*, 2000, 191-197.
[10]. C.Cabrera, F.Lloris, R. Gimenez, M. Olla, C.Lopez, Mineral content in legumes and nuts: contribution to the Spanish dietary intake. Sci. Total Environ. *308*, 2003, 1-14.
[11]. Brasil.Ministério da Saúde. Estrutura de mercado do segmento de fitoterápicos no contexto atual da indústria farmacêutica brasileira. Brasília: Ministério da Saúde, Brazil, 2007.
[12]. ANVISA. Agência Nacional de Vigilância Sanitária. Resolução – RDC n º 17, de 24/02/2000.
[13]. N. Lewen, The use of atomic spectroscopy in the pharmaceutical industry for the determination of trace elements in pharmaceuticals.J. Pharm. Biom. Anal.*55*, 2011, 653-661.
[14]. BRASIL.Farmacopeia Brasileira, Agência Nacional de Vigilância Sanitária (ANVISA), volume 1, 5 ed. FIOCRUZ: Brasília, 2010, p.172.
[15]. Moine, I.RecherchedesMétauxlourdsdanslesmédicaments,Edutedesprocedésdécrits a lapharmacopéeeuropéenne , 2éme édition, DepartmentAnalyseRhõne-PoulencSanté. Centre de Recherche de Vitry, France, 1988, p. 31-55.
[16]. T. Wang, J. Wu, T. Hartman, X. Jia, R.S. Egan, A multi-element ICP-MS survey method as an alternative to the heavy metals limit test for pharmaceutical materials. J. Pharm. Biom. Anal.*23*, 2000, 867-890.
[17]. R.N. Rao, M.V.N.K. Talluri, An overview of recent applications of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurities in drugs and pharmaceuticals.J. Pharm. Biom. Anal.**43**, 2007, 1-13.
[18]. J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, A.G. Gilman, Good and Gilman's the pharmaceutical basics of therapeutics, 9 ed., McGraw-Hill: New York, USA, p. 3-63,1999.
[19]. O.E.Orisakwe, J.K. Nduka, Leadand cadmiumlevels of commonly administered pediatric syrups in Nigeria: A public health concern? Sci. of Total Environ. *407*, 2009, 5993-5996.
[20]. K. Kordas,R.L. Canfield, P. Lopez, J.L.Rosado,G.G.Vargas, M.E. Cebrian, J.A. Rico, D. Ronquillo, R.J.Stoltzfus, Deficits in cognitive function and achievement in Mexican first-graders with low blood lead concentrations. Environ. Res. *100*, 2006, 371-386.
[21]. B.P. Lanphear, R. Hornung, J. Khoury, K. Yolton, P. Baghurst, D.C. Bellinger, R.L.Canfield, K.N. Dietrich, R. Bornschein, T. Greene, S.J. Rothenberg, H.L. Needleman, L. Schnaas, G. Wasserman, J. Graziano, R.Roberts, Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ. Health Perspect.*113*, 2005, 894-899.
[22]. J.F. Kauffman, B.J.Westenberger, J.D.Robertson, J. Guthrie, A. Jacobs, S.K. Cummins. Lead in pharmaceutical products and dietary supplements. Regulatory Toxicol.And Pharm.48, 2007, 128-134.
[23]. ATSDR (Agency for Toxic Substances and Disease Registry).Toxicologic profile for lead: update. Atlanta, GA, USA: US Department of Health and Human Services, 1999.
[24]. CDC. Preventingin lead poisoning in young children: a statement from the CDC, Atlanta, GA. CDC, p. 1-2,1991.
[25]. L.S. Miller, H.S.Koplewicz, R.G. Klein, Teacherratings of hyperactivity, inattention, and conduct problems in preschoolers. J. Abnorm. Child.Psychol.*25*,1997, 113-119.
[26]. Q. Wang,H.H. Zhao, J.W. Chen, K.D. Gu,Y.Z. Zhang, Y.X. Zhu, Y.K. Zhou, L.X. Ye, Adverse health effects of lead exposure on children and exploration to internal lead indicator. Sci. of Total Environ. *407*, 2009, 5986-5992.

[27]. B. Hina, G.F. Rizwani, H. Shareef, M.Ahmed, Atomic absorption spectroscopic analysis of some pakistani herbal medicinal products used in respiratory tract infections. Pak. J. Pharm. Sci. *25*, 2012, 247-253**.**
[28]. J.B.B. Silva, M.B.O. Giacomelli, A.J. Curtius, Iridium andrhodium as permanent chemical modifiers for the determination of Ag, As, Bi, Cd and Sb by electrothermal atomic absorption spectrometry. Microchem. J.*60*, 1998, 249-257.
[29]. J.B.B. Silva,M.A.M. Silva, A.J. Curtius, B.Welz, Determination of Ag, Pb and Sn in aqua regia extracts from sediments by electrothermal atomic absorption spectrometry using Ru as permanent chemical modifier. J. Anal. At.Spectrom.*14*, 1999, 1737-1742.
[30]. Statistica6.0 for Windows, StatSoft, Inc.,2300 East 14th Street, Tulsa, OK 74104, USA,2001.
[31]. InmetroDOQ-CGCRE-008: Instituto Nacional de Metrologia, Normalização e Qualidade, Industrial. Orientações sobre validação de métodos de ensaios químicos, 2011.
[32]. EPA.ENVIRONMENTAL PROTECTION AGENCY. Guidance for Methods Development and Methods Validation for the RCRA Program SW-846 Methods, 1992.
[33]. AOAC. Associationof OfficialAnalytical Chemists. Peer-verified Methods Program, Manual on Policies and Procedures, Arlington, VA, USA, 1993.
[34]. A. Sales, A. Alvarez, M.R. Areal, L. Maldonado, P. Marchisio, M. Rodríguez, E. Bedascarrascure, The effect of different propolis harvest methods on its lead contents determined by ET AAS and UV-vis. J. of Hazard. Mat.*37*, 2006, 1352-1356.
[35]. I.Z. Nkeiruka, O.O. Ebere, O.W.Atuboyedia, Nigerian herbalremedies andheavy metals: violation of standardrecommended guidelines. Asian Pacific J.Tropic.Biomed.2012, S1423-S1430.
[36]. A.Ting,Y. Chow, W.Tan, Microbial and heavy metal contamination in commonly consumed traditional chinese herbal medicines. J. Tradition. Chin. Med.*33*, 2013, 119-124.
[37]. E.C. Lima, F.J. Krug, M.A.Z. Arruda, Direct determination of lead in sweet fruit-flavored powder drinks byelectrothermal atomic-absorption spectrometry. Spectrochim.Acta Part B *53*, 1998, 601-611.
[38]. H. Greim, R. Snyder, Toxicology and Risk Assessment: A Comprehensive Introduction, 1 ed, John Wiley & Sons Ltda.: Chichester, England, pp. 542, 2008.
[39]. E. Obi,D.N. Akunyili, B.Ekpo, O.E.Orisakwe, Heavymetal hazards of Nigerian herbal remedies. Sci. Total Environ.*369*, 2006, 35-41.

List of Tables

Table 1.Operational conditions by GF AAS used in the analysis.

Hollow cathode Pd lamp (Varian®)	
Wavelength	283.3nm
Slit	0.5nm
Current	7.0mA
Method	
Instrumentmode	Absorbance
Calibrationmode	Scaleexpansion
Measurementmode	Peakheight
Numberofreplicatereadings	3
Maximum relative standard deviation tolerated between replicates	10%
Sample volume injected into the graphite furnace	20µL
Injectiontemperature	90°C
Rate ofinjectionspeed	5

Table 2.Program temperatures and times of the graphite furnace as recommended by the manufacturer.

Phase	**Step**	**Temperature (°C)**	**Time (s)**
Drying	1	85	5
	2	95	40
	3	120	10
Pyrolysis	4	400	5
	5	400	1
	6	400	2
Atomization	7	2100	0.9
	8	2100	2
Cleaning	9	2100	2

Table 3.Factorial 2^{4-1}planningfor determining Pb inherbal syrups.

Pyrolsistemperature(°C)	**Pyrolsis time (s)**	**Atomizationtemperature (°C)**	**Chemicalmodifier**
(-) 700	(-) 10	(-) 1900	(-) Ir
(+) 1200	(-) 10	(-) 1900	(+) Ta
(-) 700	(+) 40	(-) 1900	(+) Ta
(+) 1200	(+) 40	(-) 1900	(-) Ir
(-) 700	(-) 10	(+) 2500	(+) Ta
(+) 1200	(-) 10	(+) 2500	(-) Ir
(-) 700	(+) 40	(+) 2500	(-) Ir

(+) 1200	(+) 40	(+) 2500	(+) Ta

Table 4.Results of the reference sample readings for selection of the best modifiers employing univariate optimization.

Chemicalmodifier		Meanabsorbance	Mean background	RSD (%)	Relationabsorbance/background
Permanent	No modifier	0.3423	1.8353	10.1	0.19
	Irídio	0.3201	0.1075	4.2	2.98
	Nióbio	0.3433	1.6122	1.2	0.21
	Ruthenium	0.2699	0.0734	4.8	3.68
	Rhodium	0.3495	0.4870	5.9	0.72
	Tantalum	0.4044	0.1169	1.9	3.45
	Tungsten	0.3036	0.1411	16.9	2.15
Em solução	Palladium	0.1345	0.1785	4.6	0.75

RSD =Relative standard deviation

Table 5.Optimized furnace program for the determination of Pb in the syrup matrix using Ta as a permanent modifier.

Phase	Step	Temperature (°C)	Time (s)
Drying	1	100	15
	2	100	20
	3	120	10
	4	120	20
	5	200	10
	6	200	20
	7	400	10
	8	400	30
Pyrolsis	9	700	10
	10	700	50
Atomization	11	1900	0.8
	12	1900	2.0
Cleaning	13	2300	2.0

Table 6.Parameters of merit of the valid method for determination of Pb in syrup: linearity, limit of detection, quantification and mass characteristic.

Parameter	Value
Linear regressionequation(n=3)	Abs = (0.014 ± 0.0012)C_{Pb}+ (0.0016 ± 0.0022)
R^2 (n=3)	0.9992 ± 0.0006
Linear range (µg L^{-1})	0 – 28
Detection limite (µg L^{-1})	1.6
Quatificationlimit (µg L^{-1})	5.5
Characteristicmass (pg, n=5)	5.3 ±0.2

Mass characteristic recommended by the manufacturer, Varian: 5.5 pg.

Table 7.Recovery of lead in artificially contaminated reference sample (n = 7 for each distinct level of Pb replicates).

Additionconcentration(µg L^{-1})	Recuperation (%)	Standard deviation(n=7)
1.5	102.4	10.1
3.0	106.7	7.9
5.0	104.0	7.7
15.0	99.6	6.0

Table 8.Intra-assay precision obtained for determination of lead in artificially contaminated reference sample (n = 7 replicates for each distinct level of Pb).

Concentration (µg L^{-1})	Coefficient of variation Intra-assay %
1.5	5.3
3	5.3

5	5.9
15	5.5

Table 9.Inter-assay precision obtained for determination of lead in artificially contaminated reference sample (n = 3 days with 7 different replicates for each day and for each level of Pb).

Concentration (µg L^{-1})	Coefficient de variation Inter-assay %
1.5	13.9
3	12.1
5	6.1
15	4.0

Table 10.Lead concentration (µg L^{-1}) as determined in syrup samples (n = 2 replicates of each sample and triplicate reading).

Sample	Composition	Mean Pb concentration* (µg L^{-1})
1	Honey, simplesyrup, bromeliaddye (*Ananassativus*), propolisextract	8.4 ± 1.5
2	Honey, propolis extract, watercress fluid extract (*Nasturtium offcinale*), wild lemon fluid extract (*Siparunasp*), ginger fluid extract (*Zengiberofficinale*)	11.5 ± 1.3
3	Honey, simple syrup, guaco alcoholic extract, propolis extract	15.9 ± 2.5
4	Honey, propolis extract, eucalyptus hydroalcoholic extract and angry lemon	6.6 ± 2.5
5	Watercress alcohol, guaco fluid extract, polygala fluid extract, Tolu balsam concentrate, Ipecac syrup, aconite fluid extract, sugar, honey, sodium benzoate, methylparaben solute	8.4 ± 2.4
6	Honey, propolisandbromeliad	6.8 ± 1.3

*Correctedvaluebydilution (1:5)

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Fig. 1: Pareto chart to estimate the effect of variables optimized by factorial design 2^{4-1}, using the sample artificially contaminated reference.

Fig. 2: Curves of aqueous calibration and syrup (matrix-matching calibration) for assessment of matrix effect (n = 3).

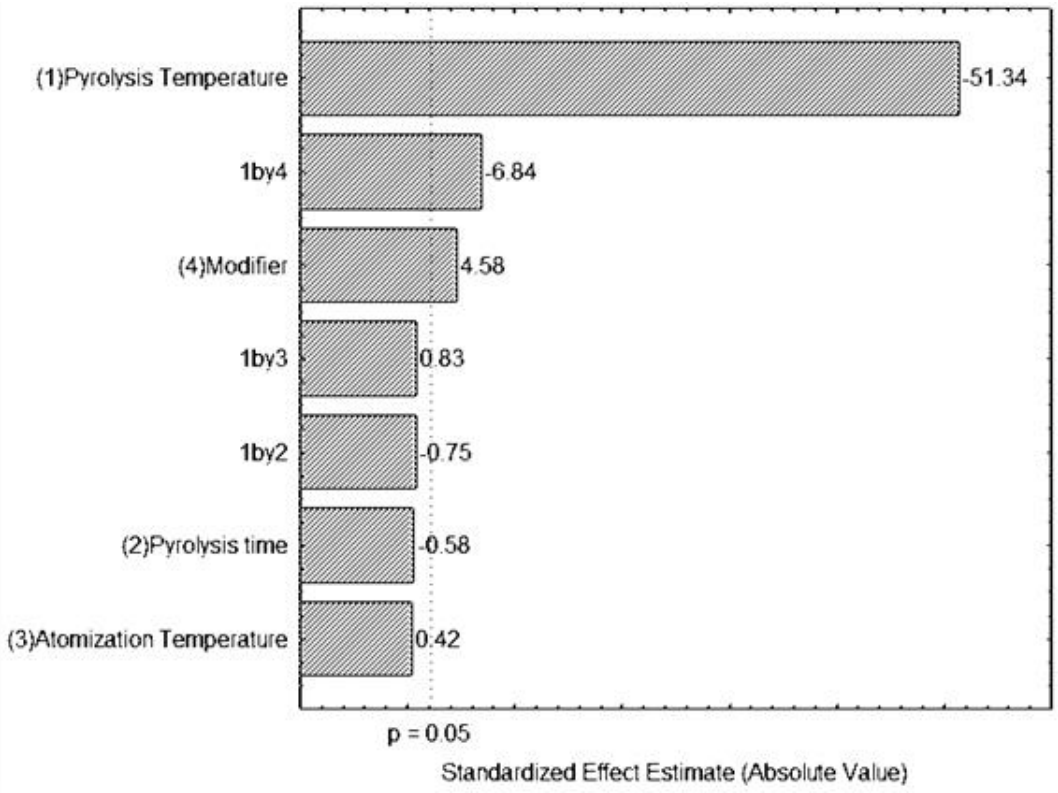

Fig. 1.

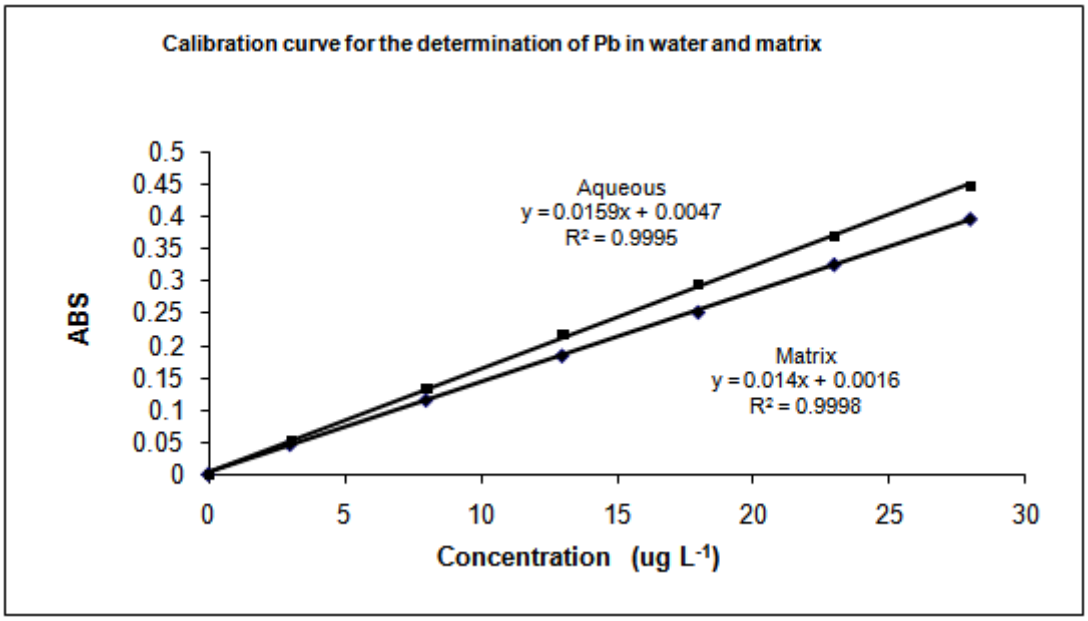
Calibration curve for the determination of Pb in water and matrix
ABS
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
Concentration (ug L-1)
Aqueous
y = 0.0159x + 0.0047
R² = 0.9995
Matrix
y = 0.014x + 0.0016
R² = 0.9998

Fig. 2.