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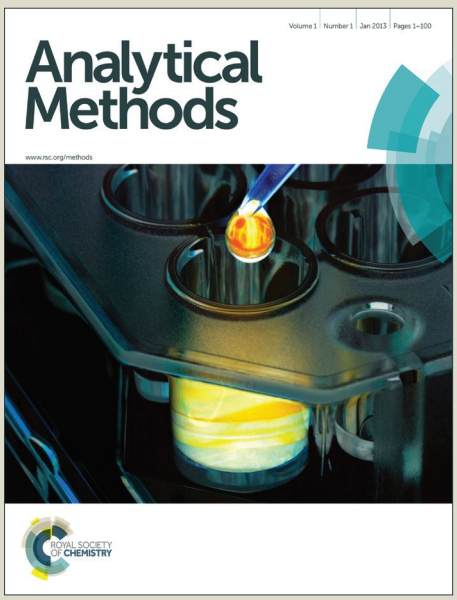

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Microwave-Assisted Digestion Using Diluted Acids for Toxic Elements Determination in Medicinal Plants by ICP-MS in Compliance to United States Pharmacopeia Requirements

Aline L.H. Muller[a], Edson I. Muller[a], Juliano S. Barin[b], Erico M.M. Flores[a*]

[a] Departamento de Química, Universidade Federal de Santa Maria, 97105-900, Santa Maria, Rio Grande do Sul, Brazil and Instituto Nacional de Ciência e Tecnologia de Bioanalítica, Campinas, SP, Brazil.

[b] Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria, 97105-900, Santa Maria, Rio Grande do Sul, Brazil

[*] Corresponding author: Tel: + 55 (55) 3220 9445

E-mail address: (Érico M. M. Flores): ericommf@gmail.com

ABSTRACT

The United States Pharmacopeia (USP) has introduced new requirements for toxic elements determination in medicinal plants. However, the digestion step is still challenging since sample preparation should provide digests fully compatible with determination technique and suitable for application in routine. The proposed method was developed for medicinal plants digestion using diluted nitric acid solutions for further determination of As, Cd, Hg and Pb by inductively coupled plasma mass spectrometry (ICP-MS) in compliance to USP chapter 2232 (*Elemental Contaminants in Dietary Supplements*) requirements. Sample masses up to 500 mg of *Passiflora incamata* L., *Paulinia cupana, Maytenus ilicifolia* and *Peumus boldus* were digested in closed vessels using HNO_3 solutions (2 to 14.4 mol L^{-1}) and also by dry ashing method recommended in current pharmacopoeias for comparison of results. As, Cd and Pb were determined by ICP-MS and Hg by flow injection cold vapor generation (FI-CVG) coupled to ICP-MS. Accuracy was evaluated using spikes and certified reference materials (CRMs) of plants (peach leaves, NIST1547 and olive leaves, BCR 062). Medicinal plants samples were digested efficiently using diluted HNO_3 solutions (4 mol L^{-1}) and agreement between 96 and 103% with CRM values was obtained for all analytes. The limits of detection (0.004 to 0.02 μg g^{-1}) were lower than those recommended by USP (0.5 to 1.5 μg g^{-1}). Significant losses in dry ashing were observed for Pb, while Hg was completely lost during digestion. Using the proposed microwave-assisted digestion method, up to eight samples could be simultaneously digested using diluted HNO_3 solution (4 mol L^{-1}) that provides digests with carbon content lower than 320 mg L^{-1}. In this sense, reagent consumption and waste generation were minimized and these features are in agreement with the green chemistry recommendations.

Keywords: Heavy metals determination; Sample preparation; Herbal medicine; Pharmacopoeia; Plant analysis; Inductively coupled plasma mass spectrometry.

1. Introduction

It is estimated that about 25% of the drugs prescribed worldwide are derived from plants which have been integrated to all systems of traditional medicine.[1] In low and middle income countries, medicinal plants are often the main source of health care. In last few decades, the use of herbal products has increased also in developed countries partially due to widespread assumption that "natural" implies "harmless".[2] However, plants are not completely free from side effects and adulteration by synthetic materials and also contamination with toxic elements, as arsenic, cadmium, mercury and lead are among the main problems related to phytotherapy.[3, 4-7] In this sense, the accurate determination of toxic elements is important to prevent the use of contaminated medicinal plants[8] and the development of analytical methodologies suitable to fulfill this requirement has attracted widespread interest.[9] According to the United States Food and Drug Administration (FDA), herbs and botanical products are considered as dietary supplements that can help to ensure an adequate dietary intake of essential nutrients and may help to reduce the risk of diseases.[10] In this sense, medicinal plants were considered as dietary supplements in manuscript and limits of elemental contaminants proposed in USP chapter 2232 were adopted.[11]

In the literature, several methods have been proposed for determination of toxic elements in plants using atomic absorption spectrometry, [12-16] inductively coupled plasma optical emission spectrometry (ICP-OES),[17-20] anodic stripping voltammetry[21] and X-ray fluorescence spectrometry.[22] More recently, the use of inductively coupled plasma mass spectrometry (ICP-MS) has increased for the determination of toxic elements in medicines due to its high sensitivity and multi element determination capability even at trace and ultra trace concentrations.[3, 23-30]

However, for the majority of analytical techniques, and especially for ICP-MS, an efficient sample digestion step is strongly required before determination, mainly for organic samples such as medicinal plants. Digestion allows the destruction of sample matrix and reduces

the risk of interferences in determination step. Digestion in open crucibles using a muffle furnace (dry ashing) is frequently recommended in many pharmacopoeias (e.g. International, European and British Pharmacopoeias) due to its high digestion efficiency and to the few requirements related to the use of simple instrumentation normally found in laboratories. However, dry ashing involves the use of high temperatures (e.g., 500 °C or more) that can result in losses of volatile elements.[31-32] Moreover, sample preparation in muffle furnaces is prone to contamination due to the ashing aids and also to the muffle environment. Using dry ashing procedures described in pharmacopeias, Lewen *et al.*[33] and Barin *et al.*[34] found losses of several elements (e.g., As, Cd, Pb and Hg) during digestion of pharmaceutical compounds and excipients. Similar results were also observed by Lira *et al.* for povidone and crospovidone raw materials.[32]

On the other hand, wet digestion has been considered as a suitable alternative to dry ashing procedures and concentrated inorganic acids (e.g., HNO_3, H_2SO_4) have been proposed using both open and closed systems.[35] Acid digestion performed in open vessels is relatively inexpensive and can be easily automated. However, some drawbacks are observed, such as low digestion efficiency that is limited by the boiling temperature of the corresponding acid or acid mixture (even using condensers), high risk of contamination through laboratory air and the use of large amounts of reagents resulting in high amount of residues generations and high blank values. In addition, the risk of losses of volatile trace elements (as Hg) is another drawback that must be considered.[36]

In order to overcome these limitations, microwave-assisted digestion using closed vessels has been widely used during last years due to the relatively higher efficiency of digestion, minimization of losses of volatile elements and reduction of blank values. In addition, the time involved could be significantly reduced and an efficient digestion could be obtained generally in less than one hour. Based on these advantages such systems have been considered as one of the best solutions for clean and fast sample preparation and well suited for trace analysis.[37-40]

Following the trends for the determination of toxic elements, in recent years The United States Pharmacopoeia (USP) has proposed changes in analytical procedures for the evaluation of elemental impurities in pharmaceutical products (including medicinal plants) by using microwave-assisted digestion in closed vessels[11, 41] as an alternative to conventional digestion procedures using dry ashing.[42] However, even the USP method and the majority of works reported in literature for the digestion of medicinal plants still recommend the use of concentrated acids to perform matrix decomposition.[14, 16, 43] In a general way, the use of concentrated reagents is hazardous, requires dilution of digests before analytes determination, and generates a high volume of concentrated acids as effluents.[44-45] Thus, a reduction of the acid concentration without decreasing the efficiency of digestion is desirable and could turn the digestion procedure more suitable for routine application. Recently, some studies have been carried out in high pressure closed systems heated by microwaves using diluted of HNO_3 (2 mol L^{-1}) instead of concentrated acids.[38, 46] Similar results have been obtained for achieving an efficient organic matrix oxidation caused by the temperature gradient generated through the vessel and gas phase reactions that allows HNO_3 regeneration and the consequent possibility of using more diluted solutions.[47] Additionally, the use of diluted nitric acid solutions is in agreement with green chemistry principles that recommends alternative driving forces for chemical reactions, such as microwave irradiation and the reduction or elimination of reagents.[48]

In this sense, the present work focuses on the development of a microwave-assisted digestion procedure using diluted acid solutions in closed vessels that was applied to four medicinal plants with subsequent determination of As, Cd and Pb by ICP-MS and Hg by flow injection cold vapor generation coupled to ICP-MS (FI-CVG-ICP-MS). Operational conditions were optimized and the efficiency of digestion was evaluated based on the carbon content in digests. Accuracy was evaluated by recovery tests and also by the use of certified reference

materials (CRMs) of plants. The obtained results were also compared with those using the classical dry ashing procedure as recommended by current pharmacopoeias.

2. Experimental

2.1. Samples, standards and reagents

Experiments were carried out using four powdered samples (particle diameter lower than 150 μm) of medicinal plants (*Passiflora incamata* L., *Paulinia cupana, Maytenus ilicifolia* and *Peumus boldus)* that were purchased in a local market. The determination of As, Cd and Pb by ICP-MS and Hg by FI-CVG-ICP-MS was performed with external calibration using analytical solutions from 0.01 to 10 μg L^{-1}, prepared in 5% (m/v) HNO_3 by appropriate dilution of a multielement stock solution (10 mg L^{-1}, SCP33MS, SCP Science, Quebec, Canada). A monoelement stock standard solution of 1000 mg L^{-1} of As, Cd, Hg and Pb (Merck, Darmstadt, Germany) was used for recovery studies. CRMs of olive leaves - BCR 62 (Community Bureau of Reference, Brussels, Belgium) and peach leaves - NIST 1547 (National Institute of Standards and Technology, Gaithersburg, Maryland, EUA) were used for accuracy evaluation.

High-purity water was obtained using a Milli-Q system (Milli-Q 18.2 MΩ cm, Millipore, Bedford, MA, USA. Analytical-grade 12 mol L^{-1} hydrochloric acid (Merck, Darmstadt, Germany) and 14.4 mol L^{-1} nitric acid (Merck, Darmstadt, Germany) were purified in a sub-boiling system (Milestone, Model Duopur, Italy). Ultrapure 18 mol L^{-1} sulfuric acid (Suprapur grade, Merck) was also used. Sodium tetrahydroborate (Vetec, Brazil) solution (0.1%, m/v) was prepared in 0.1% (m/v) sodium hydroxide (Merck) solution. Standard solutions containing 20 to 500 mg L^{-1} of C were prepared by dissolution of citric acid (Merck) for carbon content determination by ICP OES. A 0.1 mol L^{-1} potassium hydroxide (Merck) solution was used for residual acidity determination.

2.2. Instrumentation

A Multiwave 3000 model microwave sample preparation system (Anton Paar, Graz, Austria) equipped with high-pressure quartz vessels (XQ - 80, internal volume of 80 mL, maximum temperature and pressure of 280 ºC and 80 bar, respectively) was used for microwave digestion. Pressure and temperature were monitored in each vessel by the original sensors of equipment.

The analytes As, Cd, Hg and Pb were determined after digestion using an inductively coupled plasma mass spectrometer (Perkin-Elmer-SCIEX, model Elan DRC II, Thornhill, Canada), equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a cyclonic spray chamber (Glass Expansion, Inc., West Merbourne, Australia), and a quartz torch and injector tube (2 mm i.d.). Instrumental performance optimization, including nebulizer gas flow rate, ion lens voltage and torch alignment, was carried out following the instructions of the manufacturer. The following operational conditions were used: radiofrequency power of 1250 W, plasma gas flow rate of 15 L min^{-1}, auxiliary gas flow rate of 1.20 L min^{-1} and nebulizer gas flow rate of 1.12 L min^{-1}. Isotopes monitored were ^{75}As, ^{111}Cd and ^{208}Pb.

The determination of Hg was carried out by FI-CVG-ICP-MS as described in previous works [49-50]. The determination of Hg using CVG-ICP-MS system was preferred because it minimizes problems related to memory effects and results in an enhancement of sensitivity in comparison with conventional nebulization system.[51-52] The flow injection system was composed by a peristaltic pump (Gilson, Minipuls, France), a manual injector and a U-type gas-liquid separator. Tygon® tubes with internal diameter of 1.14 mm were used to carry both 0.1% (m/v) $NaBH_4$ and 1.0 mol L^{-1} HCl solutions, whereas a tube with internal diameter of 1.69 mm was used to carry water. Water was used as sample carrier and sample was mixed with 1.0 mol L^{-1} HCl using a T-type connector (0.8 mm i.d.) and carried to another T-type connector (0.8 mm i.d.) where $NaBH_4$ solution was also on-line mixed. The mixture was pumped to the gas-liquid

separator and Hg was determined by ICP-MS. The following operational conditions were used: radiofrequency power of 1250 W, plasma gas flow rate of 15 L min^{-1}, auxiliary gas flow rate of 1.2 L min^{-1} and nebulizer gas flow rate of 1.20 L min^{-1}. The isotope monitored was ^{202}Hg and sample loop of 500 µL was used.

An ultrasonic probe (VCX 130 PB, 130 W, 20 kHz, Sonics and Materials Inc., Newtown, CT, USA) was used to remove the volatile carbon compounds from sample solution before carbon determination by inductively coupled plasma optical emission spectrometry (ICP-OES) using an axial view configuration spectrometer (Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany).[53] Sonication procedure was carried out after dilution of the digested sample solution (final volume of 25 mL) during 2 min. Solution nebulization was performed using a cross-flow nebulizer coupled to a Scott double pass type spray chamber. Argon (99.996%) from White Martin-Praxair (São Paulo, SP, Brazil) was used for ICP-MS determination of toxic elements and ICP OES determination of C. The following operational conditions were used in ICP OES: radiofrequency power of 1600 W and argon flow rates of plasma, auxiliary and nebulizer of 14, 1 and 1.05 L min^{-1}, respectively. The selected wavelength for C was 193.091 nm.

Residual acidity was obtained using an automatic titration system (Titrando 836, Metrohm, Herisau, Switzerland) equipped with a magnetic stirrer (module 803 Ti Stand), 20 mL burette (Dosino 800) and pH electrode (LL Electrode plus, model 6.0262.100). Residual acidity was expressed in percentage and represents the amount of HNO_3 remaining in sample digested solutions. A muffle furnace (LF0913 Model, Jung Furnace, Blumenau, SC, Brazil) was used for dry ashing digestion.

2.3. Dry ashing digestion method according to USP

Two plants (*Passiflora incamata* L. and *Paulinia cupana*) were decomposed using the Method II described in chapter 231 from USP 37th.[42] This procedure is very similar to the other ones described in several pharmacopoeias and therefore it was used for comparison with the proposed procedure. Samples (around 4 g) were placed into a platinum crucible and sulfuric acid was added on the powdered plants. Further, samples were carefully heated until complete charring. Concentrated nitric acid (2 mL) and 5 drops of concentrated sulfuric acid were added to carbonized mass and the crucible was heated cautiously until white fumes no longer were evolved. Samples were then heated in muffle furnace between 500 and 600 ºC at a time (no longer than 2 h), until the organic matter has been completely decomposed. After cooling, 5 mL of 6 mol L^{-1} hydrochloric acid were added to the samples and crucible was heated on a steam bath for 10 min. Digests were diluted with water to 25 mL.

2.4. Microwave-assisted wet digestion in closed vessels

Passiflora incamata L. and *Paulinia cupana* samples were used for evaluation of digestion efficiency of medicinal plants samples using HNO_3 diluted solutions. Masses between 300 and 500 mg were inserted into the quartz vessels and 6 mL of HNO_3 solution (2, 4, 7, 10 or 14.4 mol L^{-1}) were added. After addition of reagents the rotor was placed inside the oven and the microwave heating program was started by applying (a) 1400 W by 20 min as ramp time up to 200 °C, (b) 1400 W for 20 min and (c) 0 W for 20 min (cooling step). After digestion, the pressure of each vessel was carefully released and the resultant solution (about 6 mL of residual solution obtained from sample decomposition using 2, 4, 7, 10 or 14.4 mol L^{-1} HNO_3) was diluted with water up to 25 mL for further ICP-MS analysis. In the present work, each run was performed with eight digestion vessels. Cleaning of vessels was carried out with 6 mL of 14.4 mol L^{-1} HNO_3 in microwave oven at 1400 W for 10 min and 0 W for 20 min for cooling. Final digests were evaluated by ICP-MS for As, Cd and Pb determination, FI-CVG-ICP-MS for Hg

determination and ICP OES for carbon determination. The concentration of carbon was expressed as mg L^{-1} of C in digests filled up to 25 mL. In order to minimize memory effects during carbon determination the wash out time was selected to 5 min in order to assure the cleaning of nebulizer and spray chamber devices. Additionally, carbon standard solution was analyzed after each digested solution and significant memory effects on carbon determination were not observed. Residual acidity in digests was determined by titration.

2.5. Evaluation of accuracy, precision, limit of detection and quantification of proposed method

The proposed method was evaluated according to the parameters described in USP 37th in Chapter 233 (Elemental Impurities – Procedures).[41] The accuracy was evaluated through spike recoveries at three concentrations for each analyte. According to USP chapter 2232, the indicated maximum limit for each analyte is 1.50 µg g^{-1} for As and Hg, 0.50 µg g^{-1} for Cd, and 1.0 µg g^{-1} for Pb which corresponds to the J values used in the manuscript. Before digestion, samples (n=5) were spiked to concentrations correspondent to 0.75, 1.50 and 2.25 µg g^{-1} of As and Hg, 0.25, 0.50 and 0.75 µg g^{-1} of Cd, and 0.50, 1.0 and 1.50 µg g^{-1} of Pb. These values are related to 50, 100 and 150% of individual analytes limits (J values), respectively.

The precision was evaluated as the repeatability and intermediate precision in six spiked samples. Samples were spiked to a concentration correspondent to 1.50, 0.50, 1.50 and 1.00 µg g^{-1} for As, Cd, Hg and Pb, respectively. These values are related to 100% of individual analytes limits (J values). The LOD and LOQ were calculated using replicates of blank samples (n=10) obtained using the same procedure of digestion. LOD and LOQ were calculated using the 3s and 10s criterion, respectively.

3. Results and Discussion

3.1. Evaluation of dry ashing digestion method

Passiflora incamata L. and *Paulinia cupana* samples were digested using Method II described in chapter 231 from USP 37th [42] that is often used in several pharmacopoeias and some difficulties were found using this procedure. A vigorous and exothermic reaction was observed immediately after the addition of acids resulting in partial losses caused by sample projection. The problem was solved by the gradual addition of reagents that spent at least one hour to be carried out. Solid particles were observed in digests and a centrifugation step was necessary before the determination by ICP-MS. Therefore, the dry ashing procedure was considered as time-consuming and needing constant analyst supervision to be properly performed. However, the most critical drawback was the poor recovery for the studied analytes, especially for Hg. Recovery values lower than 2% for Hg were obtained for *Passiflora incamata* L. and *Paulinia cupana*. On other hand, recovery assays were performed through additions of each analyte and recoveries of 70 to 83% and 92 to 98% were observed for Pb and Cd, respectively. Recoveries for As were not evaluated due to interference on the mass/charge ratio of arsenic (75 m/z) related to the high amount of hydrochloric acid used in this procedure (interferences from Cl on As species).[54]

3.2. Evaluation of microwave-assisted wet digestion in closed vessels

Concentrated nitric acid was used for the digestion of sample masses of 300, 400 and 500 mg (n = 5) and, as expected, clean digests were obtained for all sample masses studied and 500 mg was chosen for subsequent studies using diluted acids. When a 2 mol L^{-1} HNO_3 solution was used few suspended solid residues were observed in digests indicating the incompleteness of digestion. However, it was not observed for higher HNO_3 concentration and the concentration of carbon obtained after digestion using 4, 7, 10 and 14.4 mol L^{-1} HNO_3 solutions was lower than

320 mg L^{-1} showing that a high efficiency of digestion could be observed even using diluted acids (Figure 1).

The mechanism involved in microwave-assisted digestion using diluted HNO_3 was recently demonstrated and derived from the temperature gradient inside the reaction vessel during the initial steps of sample digestion and also to the presence of oxygen in the gas phase.[40, 46-47, 55] In these conditions, a regeneration reaction of HNO_3 was observed allowing the use of diluted solutions without decreasing digestion efficiency.

The residual acidity was also determined after digestion of medicinal plants and, as expected, a decrease of residual acidity was observed. Digests of *Passiflora incamata* L. and *Paullinia cupana* samples presented residual acidity lower than 33 and 22% of the original HNO_3 used in digestion, respectively, when 4 mol L^{-1} HNO_3 solution was used. The low value of residual acidity assures the compatibility of digests with ICP-based techniques (e.g. ICP-MS) and minimizes physical interferences during determination.[56]

The digests of *Passiflora incamata* L. and *Paulinia cupana* samples obtained using 4, 7, 10 and 14.4 mol L^{-1} HNO_3 were evaluated for further determination of As, Cd, Hg and Pb by ICP-MS. No significant differences were found among the results for recoveries of As, Cd, Hg and Pb in digests obtained using any nitric acid solutions (one way analysis of variance, 95% confidence level). In this sense, in order to minimize the acid consumption a 4 mol L^{-1} HNO_3 solution was chosen for subsequent experiments. It is important to mention that the matrix of evaluated samples is not the same (the used parts of *Passiflora incamata* L. and *Paulinia cupana* were leaves and fruits, respectively) showing the robustness of decomposition method even with the use of diluted acids for digestion. In this way, a similar behavior could be expected for the digestion of other medicinal plants in compliance with the requirements established in USP. Moreover, it is important to point out that the final concentration of HNO_3 solution was lower

than 4 mol L^{-1} because 6 mL of this solution was used for sample digestion and after diluted up to 25 mL.

3.3. Evaluation of precision, accuracy, limit of detection and quantification of proposed method

The proposed method was evaluated regarding precision and accuracy using *Passiflora incamata* L. sample. The accuracy of the proposed method was evaluated first by the use of recovery assays for toxic elements. Samples were spiked with reference standard solution for each analyte with 50 to 150% of *J*, where *J* is the indicated maximum limit for each analyte.[41]

All tests were performed using five replicates. Results were in agreement with the added values (Table 1) and recoveries higher than 96% were obtained for all elements.

The accuracy was also evaluated using CRMs of Peach leaves and Olive leaves (Table 2). It is important to mention that the evaluation of accuracy using CRMs is preferred, because when spikes are used the analytes could not have the same chemical form or distribution throughout the matrix. Data presented in Table 2 showed an agreement better than 95% for all the analytes when compared with certified reference values showing a suitable accuracy for the proposed method.

Precision was evaluated using repeatability and intermediate precision studies. The repeatability was evaluated by means of relative standard deviations (RSD) of measurements, and six independent samples of the material under test that were spiked with standard solutions for As, Cd, Hg and Pb at the indicated level[11] before microwave-assisted digestion. Further, digested solutions were used to the determination by ICP-MS (As, Cd and Pb) or by FI-CVG-ICP-MS (Hg). According to USP 37th, a suitable precision could be considered to a RSD lower than 20%.[41] In the present work, the obtained RSD using the proposed method ranged between 2.2 and 3.3% for all analytes (Table 3) showing its feasibility to the current trends in USP.

The intermediate precision was carried out in different days and with different analysts. Standard solutions of As, Cd, Hg and Pb were spiked in six independent samples at the indicated level corresponding to 100% of individual analytes [11] before microwave-assisted digestion. The results are shown in Table 4. The RSDs obtained were lower than 11.7% and these values are in agreement with recommended values by USP (RSD less than 25%) for this parameter.[41]

The LOD and LOQ obtained were 0.004 and 0.013 μg g^{-1} for As, 0.004 and 0.013 μg g^{-1} for Cd, 0.020 and 0.067 μg g^{-1} for Pb, respectively, and 0.007 and 0.023 μg g^{-1} for Hg. These values were at least one order of magnitude better than the limits recommended by USP and suitable for the determination of these toxic elements in medicinal plants.

3.4. Determination of As, Cd, Hg and Pb in medicinal plant samples

Four medicinal plant samples (*Passiflora incamata* L., *Paulinia cupana, Maytenus ilicifolia* and *Peumus boldus*) were digested using the optimized experimental conditions and the respective results are shown in Table 5. *Passiflora incamata* L. sample showed higher concentration of Pb (1.06 ± 0.06 μg g^{-1}) that was close to the limit proposed by USP, 1.0 μg g^{-1}, [11] showing the need of control of this element in medicinal plants. In addition, using the proposed method the blank values were always negligible for all the analytes. It should be expected as the proposed procedure involves a closed system and just the use of a diluted HNO_3 solution.

4. Conclusion

During digestion of medicinal plants using the dry ashing method losses were observed for Hg and Pb showing the limitation of this method for these analytes. The proposed microwave-assisted digestion procedure was suitable for medicinal plant digestion for further As, Cd and Pb determination by ICP-MS and also Hg by FI-CVG-ICP-MS. Using this

procedure, quantitative recoveries were obtained using a solution of 4 mol L^{-1} HNO_3. Sample masses up to 500 mg could be used under safe conditions with carbon content in digests lower than 320 mg L^{-1} that corresponds to digestion efficiency higher than 95%. Digests obtained were compatible with ICP-MS analysis and the proposed procedure was in agreement with the recommendations of green chemistry. The microwave-assisted digestion in closed vessels fulfills the pharmacopeial requirements for digestion of medicinal plants since it allows the use of diluted solutions reducing blank values and consequently the LODs and LOQs.

Acknowledgements: The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), CAPES, Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Instituto Nacional de Ciências e Tecnologia de Bioanalítica (INCTBio).

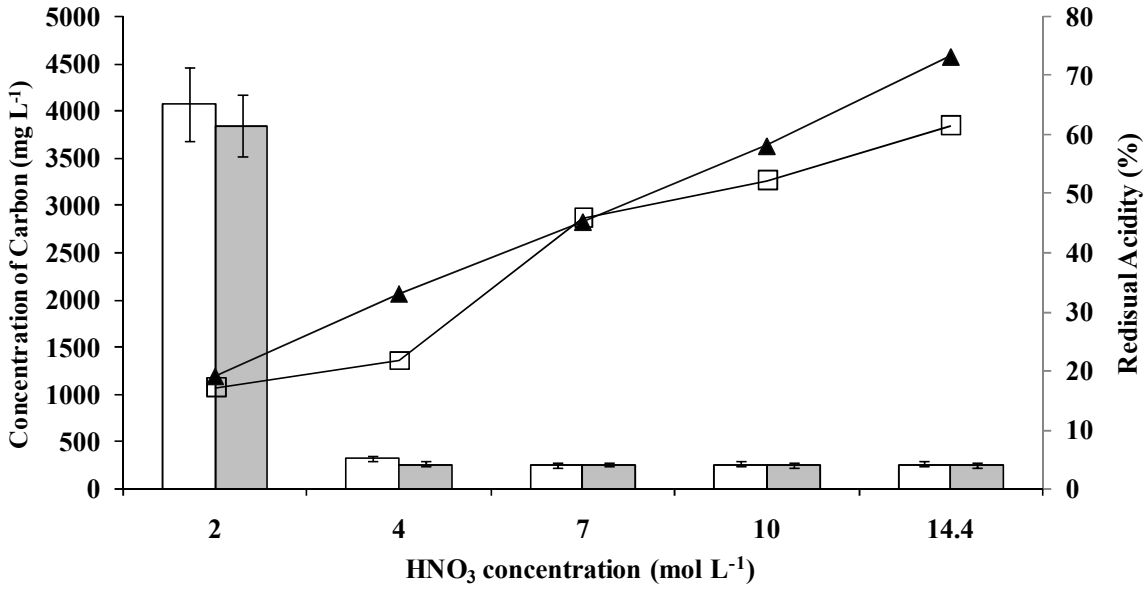

Fig. 1 Concentration of carbon (left y axis) for digestion of 500 mg of *Passiflora incamata* L. (white bars) and *Paullinia cupana* (gray bars) using HNO_3. Lines represent the residual acidity (right y axis) obtained for digestion performed to **(- □ -)** *Passiflora incamata* L. and (- Δ -) *Paullinia cupana.* Error bars represent the standard deviation (n = 5).

Table 1 - Recoveries obtained for spiked *Passiflora incamata* L. sample (50 to 150% of *J,* where *J* is the limit recommended for each element in USP; n=5, mean ± standard deviation).[11]

Analyte	Equivalent addition (μg g^{-1})	Found (μg g^{-1})	Recovery (%)
As	0.75	0.79 ± 0.03	105
	1.50	1.56 ± 0.05	104
	2.25	2.20 ± 0.01	98
Cd	0.25	0.26 ± 0.01	104
	0.50	0.51 ± 0.01	102
	0.75	0.74 ± 0.05	99
Hg	0.75	0.73 ± 0.01	97
	1.50	1.53 ± 0.02	102
	2.25	2.16 ± 0.08	96
Pb	0.50	0.48 ± 0.04	96
	1.00	1.06 ± 0.08	106
	1.50	1.58 ± 0.09	105

Table 2 - Results obtained for As, Cd, Hg and Pb determination in certified reference material of Peach leaves (NIST-1547) and Olive leaves (BCR-062) using the proposed digestion method (n=5, mean ± standard deviation).

CRM/ Elements	Certified values (μg g^{-1})	Found (μg g^{-1})
NIST-1547		
As	0.060 ± 0.018	0.064 ± 0.005
Cd	0.026 ± 0.003	0.026 ± 0.002
Hg	0.031 ± 0.007	0.030 ± 0.003
Pb	0.870 ± 0.030	0.921 ± 0.081
BCR-062		
As	0.200[a]	0.192 ± 0.011
Cd	0.100 ± 0.020	0.096 ± 0.009
Hg	0.280 ± 0.020	0.272 ± 0.021
Pb	25.0 ± 1.5	24.6 ± 2.5

[a] informed value

Table 3 - Repeatability of six independent samples spiked with standard solution containing As, Cd, Hg and Pb at the indicated level (1.5, 0.5, 1.5, 1.0 for each element, respectively).[11] Results were obtained using *Passiflora incamata* L. sample (n=6).

	Analytes (μg g^{-1})			
Sample	As	Cd	Hg	Pb
1	1.63	0.52	1.52	1.12
2	1.65	0.56	1.54	1.07
3	1.63	0.56	1.56	1.02
4	1.64	0.53	1.52	1.06
5	1.74	0.53	1.51	1.05
6	1.67	0.55	1.60	1.10
Mean	1.66	0.54	1.54	1.07
SD*	0.041	0.017	0.033	0.035
RSD (%)	2.5	3.2	2.2	3.3

*Standard deviation

Table 4 - Intermediate precision evaluated in three days using spike samples containing As, Cd, Hg and Pb at the indicated level (1.5, 0.5, 1.5, 1.0, respectively).[11] The results were obtained using *Passiflora incamata* L. sample (μg g^{-1}, n= 5) considering only the amount of analyte spiked.

	As	Cd	Hg	Pb
1º day	1.59 ± 0.05	0.49 ± 0.01	1.52 ± 0.01	0.95 ± 0.06
2º day	1.45 ± 0.04	0.58 ± 0.01	1.68 ± 0.02	1.09 ± 0.08
3º day	1.61 ± 0.03	0.53 ± 0.01	1.44 ± 0.02	1.06 ± 0.07
Mean	1.55	0.53	1.55	1.03
SD*	0.07	0.02	0.03	0.12
RSD (%)	4.5	3.8	1.9	11.6

* Standard deviation

Table 5 - Concentration of toxic elements in medicinal plants (μg g^{-1}, n=5).

Samples	As	Cd	Hg	Pb
Maytenus ilicifolia	0.032 ± 0.001	< 0.013*	< 0.023*	< 0.067*
Passiflora incamata L.	0.198 ± 0.011	0.096 ± 0.003	< 0.023*	1.06 ± 0.06
Paullinia cupana	0.017 ± 0.001	0.021 ± 0.001	< 0.023*	0.078 ± 0.005
Pneumus boldus	0.033 ± 0.001	< 0.013*	< 0.023*	< 0.067*

* LOQ

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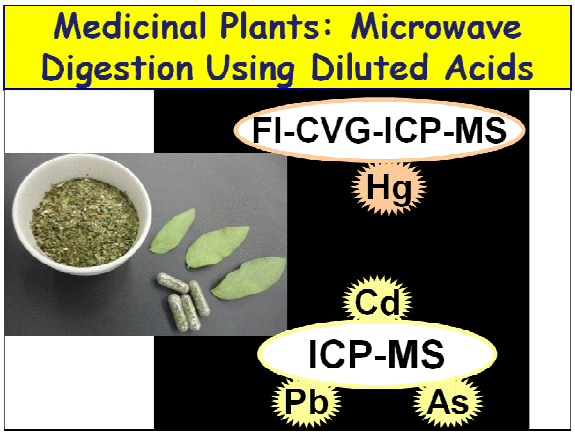

Microwave-assisted digestion of medicinal plants using diluted acid solutions in closed vessels and subsequent determination of toxic elements.