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Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry

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Abstract

Background: Traditional medicine mainly of herbal origin is widely used all around the world. Heavy metal contamination in such products is frequently reported. Accumulation of heavy metals in the human body leads to various health hazards. Thus, precise determination for such contaminants is required for safety assurance. Sample preparation is a significant step in spectroscopic analysis to achieve reliable and accurate results. Wet digestion methods are basically used for the dissolution of herbal product samples prior to elemental analysis.

Methods: This study has been designed to evaluate the efficiency of three acid digestion methods using different solvents. Five samples were digested with three different acid digestion methods namely method A (a combination of nitric-perchloric acids $\text{HNO}_3\text{-HClO}_4$ in a ratio 2:1), method B (only nitric acid HNO_3), and method C (a mixture of nitric-hydrochloric acids $\text{HNO}_3\text{-HCl}$ in a ratio 1:3), to recommend the most efficient digestion method that gains the highest analyte recovery. The analysis of arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn), and iron (Fe) was conducted using various techniques of atomic absorption spectrometry (AAS).

Results: The statistical analysis revealed that method C which represented the combination of nitric-hydrochloric acids $\text{HNO}_3\text{-HCl}$ in a ratio 1:3 was the most efficient digestion method for herbal product samples as it had given a significant high recovery ($p < 0.05$) for all metals compared to method A and method B. Accuracy of the proposed method was evaluated by the analysis of standard reference material (SRM) 1515 Apple Leaves from the National Institute of Standards and Technology (NIST) which presented good recoveries for all metals ranging from 94.5 to 108 %.

Conclusion: Method C provides highest recovery for all the analytes under investigation using AAS in herbal medicine samples.

Keywords: Traditional medicine, Acid digestion, Heavy metals, Nitric acid

Background

Traditional medicine (TM) has a significant contribution to the global health care system (Chan 2003). A significant proportion of the world's population relies on TM to support their basic health care needs (Jayaraj 2010). Therefore, safety and quality of such products become a major concern (Igweze et al. 2012). Inorganic contaminants such as heavy metals are often present in herbal medicine in various concentration levels (Saeed 2010; Hina et al. 2011; Qing-hua et al. 2001). The presence of

heavy metals in such products is either referred to the ingredients itself or they might arise during the processing part (Sharma and Dubey 2005). Arsenic, cadmium, lead, and nickel are toxic heavy metals that might be present in TM (Uddin et al. 2012). Prolonged exposure to these metals may cause many adverse health effects including cancer (Ray and Ray 2009). Although zinc and iron are essential metals for the human body at trace concentrations yet, they are toxic if present in higher concentrations (Vaikosen and Alade 2011). Consequently, heavy metal content in TM products must be accurately determined. Highly sensitive spectroscopic techniques such as flame (FAAS), graphite furnace (GFAAS), and hydride generation atomic absorption

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spectrometries (HGAAS) are mainly applied for elemental analysis in various samples. Such techniques require aqueous samples. Thus, solid samples need to be regularly converted into solutions using an appropriate dissolution method (Charun and John 2006). Acid digestion methods are generally used for the dissolution of herbal product samples prior to elemental analysis (Duyusen and Görkem 2011). In a spectroscopic elemental analysis sample preparation, acid digestion is an important step of the entire analytical procedure. It has a substantial effect on the recovery of various analyte contents in highly complex matrices such as herb and plant materials. Therefore, it requires further improvement to provide a standard technique that is able to gain accurate results (Nabil 2010). It is essential to assess the digestion efficiency of various digestion methods to achieve the optimal sample preparation method with clearer background (low noise level). Majority of samples are dissolved by various acids prior to spectroscopic elemental analysis. Wet/acid digestion has the benefits of being effective on both organic and inorganic substances as it has the ability to destroy the sample matrix and consequently minimize the interference. However, at this preliminary stage of the analytical processes, there are still some sources of potential errors such as incomplete digestion. Rational selection of the acid combinations used for various sample digestions is very important to achieve the reliable analytical method. Nitric acid is often utilized for this purpose as an oxidant reagent either individually or mixed with other digestion reagents such as acids and/or hydrogen peroxide. The oxidizing capacity, accessibility, and the affordability of nitric acid make it prevalent in this respect (Sastre et al. 2002). This study was aimed to assess the digestion efficiency of three acid digestion methods namely A, B, and C which represented a combination of nitric-perchloric acids $\text{HNO}_3\text{-HClO}_4$ in a ratio of 2:1, only nitric acid HNO_3 , and a mixture of nitric-hydrochloric acids $\text{HNO}_3\text{-HCl}$ in a ratio of 1:3, respectively. Five TM samples of herbal origin were digested with the abovementioned methods. The digestion processes were conducted using the conventional open vessel heating system as it provided the advantage of low equipment cost (Güler and Arzu 2006). The analysis of heavy metals was conducted using various techniques of atomic absorption spectrometry (AAS).

Methods

Traditional medicine samples

Finished products of traditional medicine samples were collected from three different states in the East Coast region of Peninsular Malaysia, namely Pahang, Terengganu, and Kelantan, from various commercial places of the sampling area. Finished herbal products used for medical purposes are herbal preparations that underwent all stages

of production including packaging. They might consist of different herbs/plants, various parts of the same plants, and plant extracts. Five samples of herbal origin in capsule and tablet dosage forms were used to perform the optimization of acid digestion method.

Chemicals and sample preparation

All chemicals and reagents used in this study were of analytical and trace metal grades. Trace metal grades 65 % HNO_3 , 37 % HCl , and 70 % HClO_4 were obtained from Fisher Malaysia. Stock standard solutions for each metal arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn), and iron (Fe) with a concentration of 1000 ppm were supplied by Perkin Elmer USA. Deionized water was used throughout the study. Sodium borohydride (NaBH_4), sodium hydroxide (NaOH), L-ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$), and potassium iodide (KI) were from Merck (Germany). A standard reference material (SRM) 1515 Apple Leaves was obtained from the National Institute of Standards and Technology (NIST, USA). All glassware were soaked in 5 % (v/v) HNO_3 overnight then rinsed with deionized water and dried using lab dryer FDD-720 prior to use.

Methods of digestion

Samples were accurately weighed (0.5 g each) and placed in a 100-mL PTFE beaker. The samples were subjected to three different acid digestion methods, as will be explained, to identify the most appropriate digestion method to determine the contents of As, Pb, Cd, Ni, Zn, and Fe in TM samples by AAS.

Method A (nitric-perchloric acid digestion 2:1)

To the sample, 5 mL of 65 % HNO_3 was added, and then the mixture was boiled gently for 30–45 min. After cooling, 2.5 mL of 70 % HClO_4 was added, and the mixture was gently boiled until dense white fumes appeared. Later, the mixture was allowed to cool, and 10 mL of deionized water was added followed by further boiling until the fumes were totally released (Hseu 2004).

Method B (nitric acid digestion)

To the sample, 5 mL of 65 % HNO_3 was added, and then the mixture was boiled gently over a water bath (90 °C) for 1–2 h or until a clear solution was obtained. Later, 2.5 mL of 65 % HNO_3 was added, followed by further heating until total digestion (Zheljzakov and Nielson 1996).

Method C (nitric-hydrochloric acid digestion 1:3)

To the sample, 9 mL of freshly prepared acid mixture of 65 % HNO_3 was added, and 37 % HCl was added. Then, the mixture was boiled gently over a water bath (95 °C) for 4–5 h (or until the sample had completely dissolved) (Ang and Lee 2005).

Table 1 Instrumental parameters for FAAS of Zn and Fe analysis; GFAAS for Cd, Pb, and Ni analysis; and HGAAS for As analysis

Instrumental parameters	FAAS Zn/Fe	GFAAS Cd/Pb/Ni	HGAAS As
Wavelength (nm)	213.9/248.3	228.8/283.3/232.0	193.7
Slit (nm)	0.7/0.2	0.7/0.7/0.2	0.7
Lamp type	HCL/HCL	HCL/EDL/HCL	EDL
Atomization temp. (°C)	2300/2300	1400/1500/2300	900

During the digestion procedures, the inner walls of the beakers were washed with 2 mL of deionized water to prevent the loss of the sample, and at the last part of the digestion processes, the samples were filtered with Whatman 42 (2.5- μ m particle retention) filter paper. Then, a sufficient amount of deionized water was added to make the final volume up to 50 mL.

Analytical procedure

Heavy metals were measured using a Perkin Elmer atomic absorption spectrometer (AAnalyst 800). In this study, three different AAS techniques were used for elemental measurements in certified and real samples of finished herbal products. The instrument is designed to analyze the sample using three different atomization techniques: FAAS for Zn and Fe; GFAAS for Cd, Pb, and Ni; and HGAAS for As. In FAAS, the aqueous sample is

aspirated in the flame atomizer by the nebulizer to measure the analyte concentration at a parts per million (ppm) concentration level with good precision. Pb, Cd, and Ni were analyzed by GFAAS. It is an efficient measurement system for a number of elements at relatively low levels of concentration with the use of several matrix modifiers (Shah et al. 2009). In GFAAS, the instrument is equipped with a transverse heated graphite atomizer (THGA) which provides uniform temperature distribution across the entire length of the graphite tube atomizer to overcome the potential chemical interference effects; also, the instrument offers an auto-sampler system and provides an accurate background correction (Zeeman correction). Arsenic was detected by the HGAAS method which is based on the reaction of NaBH₄ with acidified sample results in total separation of the analyte as hydride from the matrix before measurement which reduces the matrix interferences. In this technique, standards and samples were pre-reduced from an arsenate pentavalent (V) to an arsenite trivalent (III) state. This was achieved by adding a reducing solution containing 5 % (w/v) KI, 5 % (w/v) ascorbic acid, and 10 % HCl. The treated samples and standards were allowed to stand at room temperature for approximately 40 min prior to analysis. Table 1 shows the instrumental parameters of FAAS, GFAAS, and HGAAS for all metals.

Table 2 Concentration of different metals (μ g/g) (\pm SD) in traditional medicine samples (TM 1–TM 5) using different acid digestion methods

Sample ID	As	Cd	Pb	Ni	Zn	Fe
TM 1						
A	0.25 (\pm 0.02) ^a	0.25 (\pm 0.004)	4.177 (\pm 0.09)	6.26 (\pm 0.07)	51.7 (\pm 0.1)	219 (\pm 0.7)
B	0.24 (\pm 0.02)	0.19 (\pm 0.009)	4.16 (0.04)	6.30 (\pm 0.02)	46.9 (\pm 0.3)	154 (\pm 0.7)
C	0.3 (\pm 0.04)	0.33 (\pm 0.004)	4.66 (\pm 0.02)	7.02 (\pm 0.9)	56.8 (\pm 1.3)	306 (\pm 1.6)
TM 2						
A	0.25 (\pm 0.03)	0.19 (\pm 0.004)	2.545 (\pm 0.1)	4.54 (\pm 0.05)	37.6 (\pm 0.02)	213 (\pm 1.2)
B	0.27 (\pm 0.02)	0.15 (\pm 0.02)	2.41 (\pm 0.09)	4.26 (\pm 0.02)	36.2 (\pm 0.01)	100 (\pm 0.7)
C	0.33 (\pm 0.03)	0.26 (\pm 0.002)	2.95 (\pm 0.01)	4.70 (\pm 0.16)	41.5 (\pm 0.2)	303 (\pm 1.1)
TM 3						
A	0.29 (\pm 0.04)	0.12 (\pm 0.001)	4.94 (\pm 0.04)	4.60 (\pm 0.01)	11.9 (\pm 0.1)	114 (\pm 0.5)
B	0.73 (\pm 0.04)	0.11 (\pm 0.001)	4.94 (\pm 0.04)	4.64 (\pm 0.01)	11.4 (\pm 0.1)	93 (\pm 1.9)
C	1.2 (\pm 0.06)	0.14 (\pm 0.004)	5.39 (\pm 0.05)	4.89 (\pm 0.02)	14.3 (\pm 0.1)	168 (\pm 0.5)
TM 4						
A	1.2 (\pm 0.06)	0.15 (\pm 0.001)	2.48 (\pm 0.3)	4.40 (\pm 0.1)	20.4 (\pm 0.1)	227 (\pm 2.0)
B	0.85 (\pm 0.01)	0.12 (\pm 0.001)	2.32 (\pm 0.07)	4.28 (\pm 0.07)	19.5 (\pm 0.3)	198 (\pm 1.7)
C	1.9 (\pm 0.12)	0.24 (\pm 0.001)	2.93 (\pm 0.03)	4.80 (\pm 0.12)	23.1 (\pm 0.3)	688 (\pm 2)
TM 5						
A	1.3 (\pm 0.06)	0.18 (\pm 0.03)	1.95 (\pm 0.09)	5.32 (\pm 0.09)	44.2 (\pm 0.3)	108 (\pm 0.1)
B	0.88 (\pm 0.03)	0.18 (\pm 0.03)	1.88 (\pm 0.10)	5.00 (\pm 0.03)	42.3 (\pm 0.3)	92 (\pm 0.7)
C	1.6 (\pm 0.01)	0.25 (\pm 0.003)	2.50 (\pm 0.3)	5.80 (\pm 0.29)	52.3 (\pm 1)	127 (\pm 0.5)

TM traditional medicine, A nitric-perchloric acid, B nitric acid, C nitric-hydrochloric acid

^aResults presented as the mean of triplicates (\pm SD)

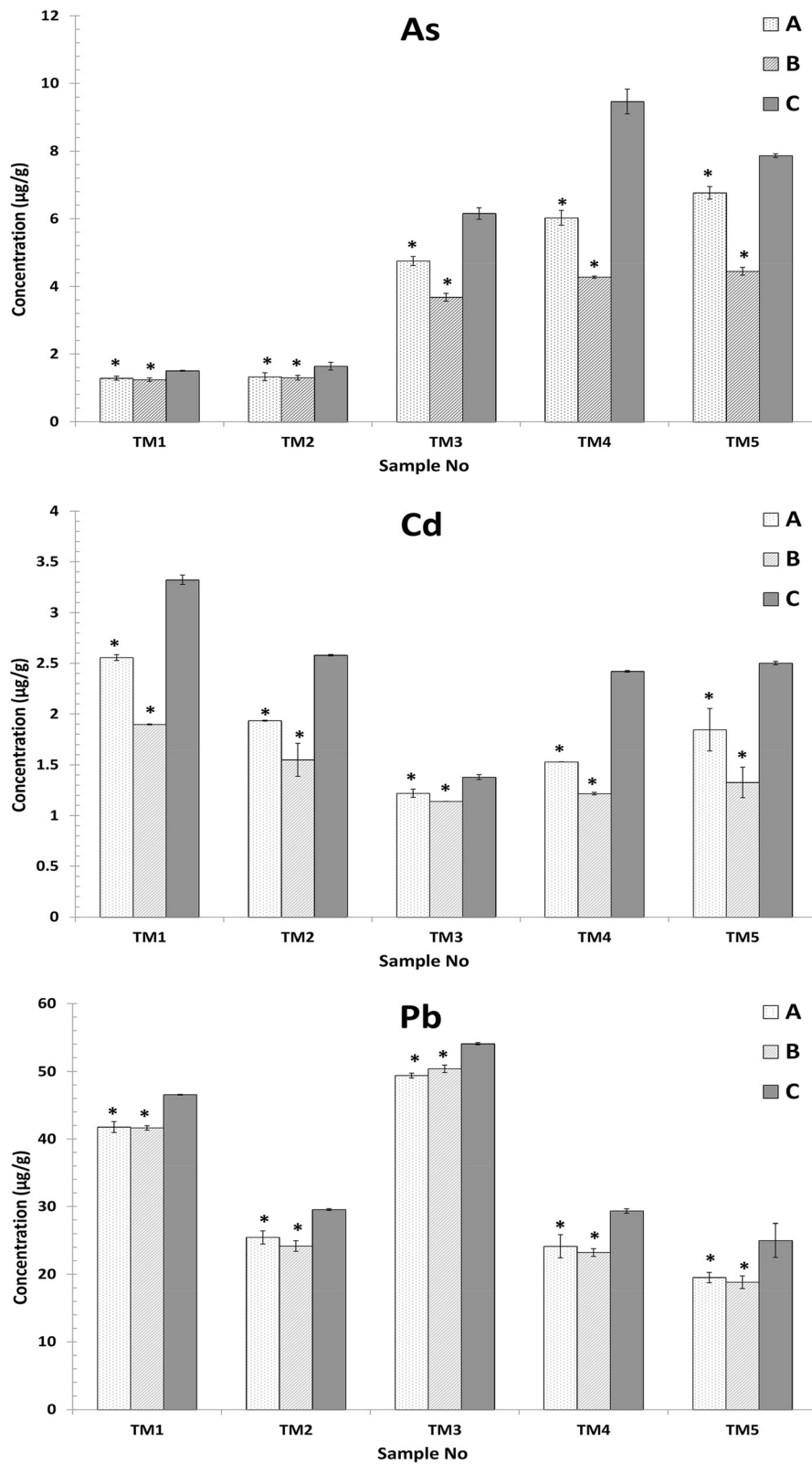


Fig. 1 Concentration of As, Cd, and Pb ($\mu\text{g/g}$) in traditional medicine samples using methods A, B, and C

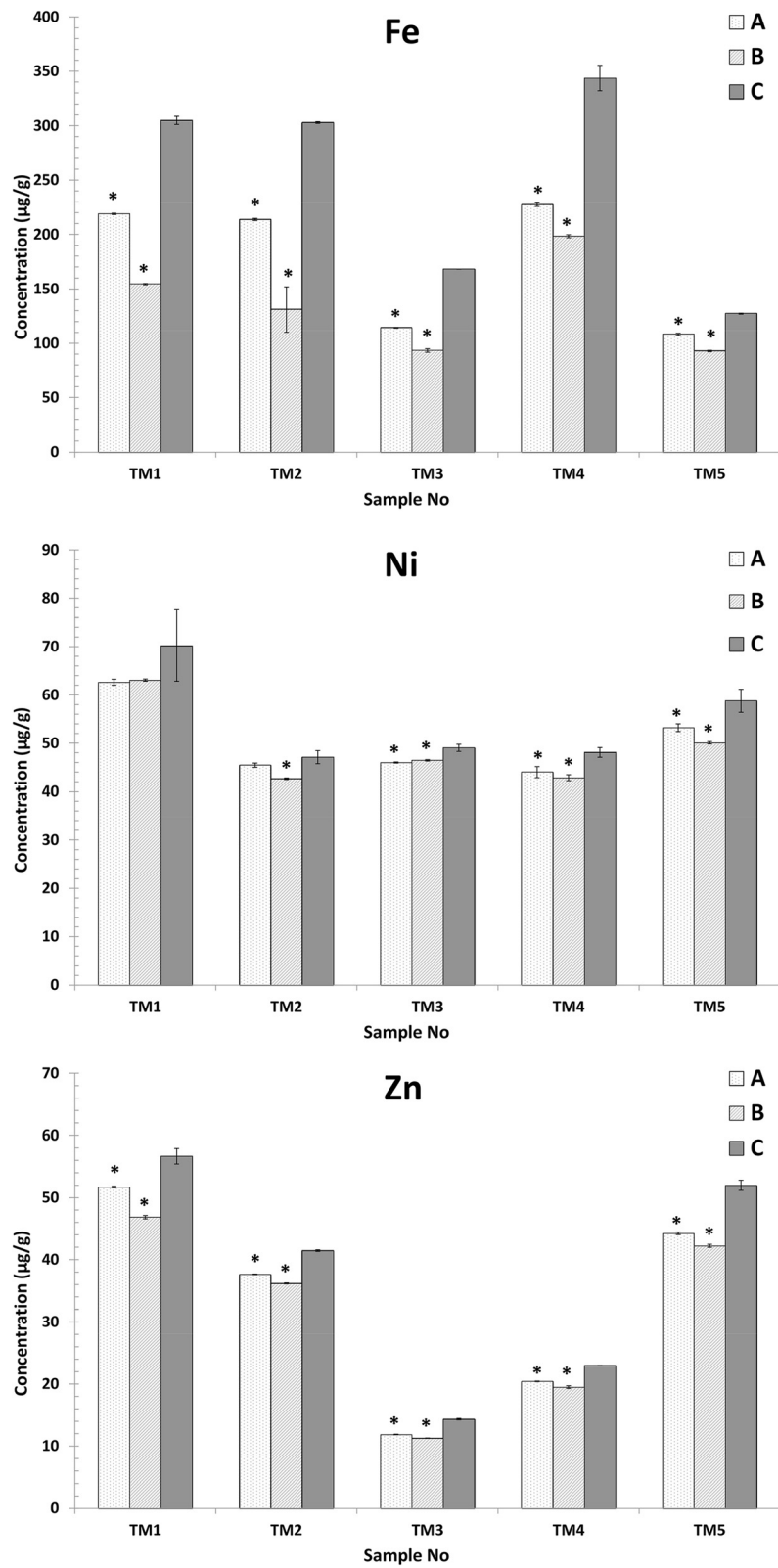


Fig. 2 Concentration of Fe, Ni, and Zn (µg/g) in traditional medicine samples using methods A, B, and C

Analysis of the standard reference material

The accuracy of the optimized method was verified by the analysis of SRM 1515 Apple Leaves.

Statistical analysis

Results were expressed as the mean of triplicates \pm standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons using SPSS.

Results and discussion

The results obtained from all experiments indicated that method C which represented the mixture of HNO_3 -HCl had given the highest analyte recovery for As, Cd, Pb, Ni, Zn, and Fe in TM samples. Method A using the HNO_3 - HClO_4 mixture and method B using HNO_3 only gave convergent recoveries for all elements. The mean values of As, Cd, Pb, Ni, Zn, and Fe with standard deviation for each method are shown in Table 2. The differences were found statistically significant with a p value <0.05 for all TM samples digested with method C for all elements (Figs. 1 and 2).

The accuracy of the proposed method was checked by the analysis of SRM 1515 Apple Leaves obtained from NIST. The results indicate good agreement between measured and certified values, and the recovery percentage for all metals was in the range 94.5–108 % within the specification limit of AOAC guidelines which verifies the accuracy of the method. Table 3 shows the results for metals content in SRM.

All the TM samples were contained heavy metals at different concentrations. Highest analyte recoveries for all TM samples were gained using method C that ranged 0.3–1.9; 0.14–0.33; 2.5–4.66; 4.7–7.02; 14.3–56.8; and 127–688 $\mu\text{g/g}$ for As, Cd, Pb, Ni, Zn, and Fe, respectively. Based on the fact that TM are highly consumed worldwide, a significant concern from many health institutes in different countries had imposed permissible limits of heavy metals in raw/finished herbal product. In Canada, the maximum limits for As, Pb, and Cd are 5, 10, and 0.3 ppm, respectively; in India, 10, 10, and 0.3 ppm for As, Pb, and Cd, respectively (Gupta et al.

2010). It is equitable to assume that heavy metal intake through such products has significant influence on human's health. Therefore, an adequate method for their determination is of importance.

Previous studies suggested various methods for digesting different samples for metal analysis (Nabil 2010). Aqua regia has been proposed as the best digestion method for samples with low carbonate or organic matter contents such as sediments and agricultural soils (Sastre et al. 2002). Another study reported that there was no significant differences between the digesting capacity of HNO_3 acid and HNO_3 - HClO_4 acid mixture in the measurement of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) contents of barley (*Hordeum vulgare* L. cv. *Minorimugi*) and rice (*Oryza sativa* L. cv. *Akihikari*) seedlings (Shaibur, et al. 2010). Nitric acid digestion was proposed as the most efficient method for recovering Cd, Mn, and Ni in the majority of composts samples (Hseu 2004). Another study recommended the combination of HNO_3 -HCl in a ratio of 1:2 as the most efficient digestion method which yielded the highest recovery of Pb, Zn, and Fe in canned sardines samples (Fong et al. 2006). However, TM samples have complex matrices as they are made from either one herb or a mixture of herbs from any part of a plant such as leaves, roots, seeds, and flowers that might have different chemical properties. The digestion capacity of hydrochloric-nitric acids HNO_3 -HCl in a ratio of 1:3 mixture had proven to be the best acid combination suitable for the decomposition of TM samples due to the ability of such mixture to release the metal ions from such complex matrices of herbal materials and subsequently to minimize the noise level during the detection procedure.

Conclusions

Sample preparation is a crucial step in spectroscopic elemental analyses as it can considerably affect the accuracy of results. Significant differences between the digesting capacities of different methods were identified. The digestion capacity using a mixture of hydrochloric-nitric acids HNO_3 -HCl in a ratio of 1:3 was the most efficient method in terms of the recovery of As, Cd, Pb, Ni, Zn, and Fe in herbal medicine samples.

Table 3 Recovery percentages and concentrations of As, Cd, Pb, Ni, Zn, and Fe in SRM (1515)

Analyte	SRM conc. $\mu\text{g/g}$ ($\pm\text{SD}$)	Measured conc. $\mu\text{g/g}$ ($\pm\text{SD}$)	Recovery (%)
As	0.038 (± 0.007)	0.039 (± 0.002)	102
Cd	0.013 (± 0.002)	0.012 (± 0.009)	96
Pb	0.47 (± 0.02)	0.5 (± 0.02)	106
Ni	0.91 (± 0.12)	0.86 (± 0.004)	94.5
Zn	12.5 (± 0.3)	13.5 (± 0.3)	108
Fe	83 (± 5)	83.3 (± 4)	100

Abbreviations

AAS: atomic absorption spectrometer; AOAC: American Organization of Analytical Chemistry; ANOVA: analysis of variance; As: arsenic; Cd: cadmium; EDL: electrodeless discharge lamp; FAAS: flame atomic absorption spectrometer; Fe: iron; GFAAS: graphite furnace atomic absorption spectrometer; HCL: hollow-cathode lamp; HCl: hydrochloric acid; HClO_4 : perchloric acid; HNO_3 : nitric acid; KI: potassium iodide; NaBH_4 : sodium borohydride; NaOH: sodium hydroxide; HGAAS: hydride generation atomic absorption spectrometer; $\text{Mg}(\text{NO}_3)_2$: magnesium nitrate; NaOH: sodium hydroxide; Ni: nickel; NIST: National Institute of Standards and Technology; Pb: lead; SD: standard deviation; SPSS: Statistical Package for Social Sciences; SRM: standard reference material; Zn: zinc.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

RSK, ABM HU, MA has conducted the experimental part and manuscript preparation. AA, SAA has contributed for the statistical analysis and AK and SAA has edited the paper for technical improvement.

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