Original Article

Comparison of Digestion Methods for the Determination of Trace Elements and Heavy Metals in Human Hair and Nails

Ismarulyusda Ishak, Farah Dayana Rosli, Jamaludin Mohamed, Muhammad Faiz Mohd Ismail

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Biomedical Science Programme, Faculty of Health Sciences, School of Diagnostic and Applied Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Abstract

Background: **Microwave is the most reliable sample digestion method. However, it requires expensive microwave digester automation and has relatively low productivity. In this study, three non-automated digestion methods, i.e. wet acid digestion using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), wet acid digestion using HNO_3, and dry washing, are compared in order to determine the best approach.**

Methods: **Certified reference material IAEA-086 (International Atomic Energy Agency, Austria) and hair and nail samples from 20 female students of Universiti Kebangsaan Malaysia, aged 19 to 30 years, were collected and analysed using the three digestion methods.**

Results: **For hair samples, analysis of variance of repeated measures showed significant differences in the level of all elements ($P < 0.001$) between the three methods. For nail samples, only the copper (Cu) level showed no significant difference ($P = 0.100$) between methods. Wet acid digestion using HNO_3 and H_2O_2 showed the best within- and between-run relative standard deviation (RSD) values, with within-run RSD for all elements, except for selenium (Se), < 5%. The between-run precision ranges from 6.14% to 17.96% for hair and from 3.53% to 11.52% for nail samples. Wet acid digestion using HNO_3 and H_2O_2 showed both good accuracy and precision for manganese (Mn) and magnesium (Mg), with percentage recoveries of 110% and 96.9%, respectively. All elements show higher method detection limit (MDL) values than the previous study: 0.05 µg/g Mg for wet acid digestion using HNO_3, 0.02 µg/g Se for wet acid digestion using HNO_3 and H_2O_2, and 0.2 µg/g Mg for dry ash method.**

Conclusion: **Wet acid digestion using HNO_3 and H_2O_2 proved to be the best method in terms of precision, accuracy, recovery, and MDL. However, only Mn and Mg showed adequate precision, accuracy, and percentage of recovery.**

Keywords: *trace element, heavy metal, digestion, hair, nails*

Introduction

Copper (Cu), selenium (Se), manganese (Mn), magnesium (Mg), and zinc (Zn) play important roles in human biochemical processes, and their deficiency and excess have serious impacts on human health (1). Thus, determination of the concentration of these elements in biological samples is useful for nutritional assessment and for the identification and monitoring of environmental and occupational exposures, as well as in the diagnosis and monitoring of diseases (2,3). For the determination of trace elements, hair and nail analysis provides several advantages over blood and urine testing, including the easy collection, transportation, and handling of samples, need for no special storage conditions, high concentration of elements, non-invasive procedure, longer retrospective time frame (months) representation, and lower costs (4–10). Inductively coupled plasma-mass spectrometry (ICP-MS) is suggested to be the most suitable analytical technique for ultra-trace multi-element analysis, with high sensitivity and ability to measure a large range of concentrations (9,11,12). Various digestion methods have been reported, such as wet acid, dry ash, and microwave acid digestion, which can be appropriately modified. Each method has its own advantages and disadvantages (12,14). According to Bass et al. (9), microwave digestion is the most appropriate method for standardisation. However, it requires an expensive microwave digester (15) and it has relatively low productivity (16), suitable

for diagnostic investigations only. A method providing high productivity and reliable results is therefore desirable. Ming and Bing (13), reported that the dry ash, wet acid, and microwave digestion methods showed no statistical differences in accuracy.

A review by Rodushkin and Axelsson (6), describes the numerous attempts to determine trace elements in hair and nail samples and to compare digestion methods; however, a full analytical performance evaluation has not yet been reported. Analytical performance results, including accuracy, precision, percentage of recovery, and method detection limit (MDL), will ensure the validity of the outcome and are useful for comparison with other studies. Thus, the aim of this work is to compare the analytical performance of the three non-automated digestion methods of hair and nail samples using ICP-MS.

Materials and Methods

Instrumentation

All determinations were performed using Sciex Elan 900 ICP-MS instrument (Perkin Elmer, USA) under normal operating conditions (Table 1). A hot plate (Cimarec, USA), a model FD 53 drying oven (BINDER, USA), and an electric furnace (Thermolyne, USA) were used.

Reagents and standard solutions

All reagents used, including HNO_3 (65%), acetone, H_2O_2 (30%), and Triton-X 100 (Merck, Darmstadt, Germany), were of analytical grade. Deionised Milli-Q water (18.2 MΩ cm; Millipore, Bedford, MA, USA) was used. The standard solutions were prepared by dilution of the multi-element calibration standards (Perkin Elmer, Norwalk, USA).

Equipment preparation

All glass and plastic wares were soaked in 5% (v/v) analytical grade HNO_3 overnight and then rinsed with deionised Milli-Q water. The equipment is dried in the oven and appropriately stored to avoid contamination and dust.

Samples

The samples used in this study were human hair certified reference material (CRM) IAEA-086 (International Atomic Energy Agency, Austria) and hair and nail samples collected from 20 female students aged 19–30 years. Twenty pieces of hair were collected approximately one inch from the scalp, by using stainless steel scissors. Fingernails are collected using a stainless steel nail clipper. Hair and nail samples were stored in a sealed polyethylene plastic bag and were kept at room temperature until the digestion process.

Sample washing procedure

In this study, the hair and nail sample washing method proposed by the IAEA (17) was applied, with some modifications recommended by Miekeley et al. (18), and by Batista et al. (16). Approximately 1 cm of each piece of hair is cut. Any visible dirt was physically removed from the nail samples by scraping with cotton. Each sample was placed into a 50 mL beaker. The washing procedure involved stirring of the samples with different solvents in the following sequence: 0.5% Triton-X 100, deionised water, and acetone, using a mechanical shaker, followed by repeated rinsing with deionised water. The stirring time for each

Table 1: Operating conditions of the ICP-MS

ICP-mass spectrometry	
Instrument	**Perkin-Elmer Elan 900**
RF-power	1000W
Plasma gas flow rate	20 L/min
Spray chamber	Ryton double pass
Cone	Nickel
Resolution	0.7 ± 0.1 amu
Dwell time	250 ms
Sweeps	20 per reading
Replicate	Three

The normal operation condition outlined of Sciex Elan 900 Inductively Coupled Plasma Spectrometry (ICP-MS). Source : Perkin Elmer, U.S.A.

solvent was a few minutes for the hair and 1 h for the nail samples. The samples were dried in a drying oven at 60 °C overnight and were brought to room temperature before digestion or kept in clean polyethylene bags until the digestion process.

Sample digestion procedure

For each digestion method, all samples were digested for three non-consecutive days. The methods performed were optimised in terms of completeness of digestion, turnaround time, minimal contamination, simplicity, safety, and equipment used before analysis. For each digestion method, the blanks were prepared in the same way as the samples.

Wet acid digestion using nitric acid

The procedure described by Wongwit et al. (19) was adapted. Approximately 20–30 mg of hair or nail clippings is weighed and placed into a test tube. Concentrated HNO_3 (1 mL) was added, and the test tube was placed on a heating plate and heated to 100 °C. Glass marble was placed on top of each tube and the digestion was performed for 1 h. After cooling to room temperature, the solution was transferred into a graduated polypropylene tube and diluted to 10 mL with deionised water.

Wet acid digestion using nitric acid and hydrogen peroxide

In this method, the procedure recommended by Miekeley et al. (18) was followed. Approximately 20 to 30 mg of hair or nail clippings is weighed and placed into a closed, graduated polypropylene tube. HNO_3 (0.5 mL) is added and the mixture is left overnight at room temperature. The samples are kept for 1 h in a drying oven at 60 °C. After cooling, 0.2 mL of H_2O_2 was added and the samples were incubated for 1 h in a drying oven at 60 °C. The solutions are diluted to 10 mL using deionised water.

Dry ash method

The sample digestion procedure recommended by Aydin (20) was used. About 20 mg of hair or nail clippings was weighed and placed into a porcelain crucible for dry digestion. The sample was placed on a hot plate at the maximum temperature for 90 min. The bowls were then placed into a furnace and the temperature was slowly increased to 500 °C over 1 h. The sample was ashed for 4 h until a white or light grey ash residue is formed. The residue was dissolved in 1 mL of HNO_3. The solution is then transferred into a polypropylene tube and diluted to 10 mL with deionised water.

Method validation

In order to demonstrate the validity of the proposed method, the within- and between-run precision, percentage of recovery, accuracy, and MDL tests were carried out. Precision has been defined as the level of reproducibility of experimental results (30). Within-run or repeatability assesses precision during a single analytical run, whereas between-run measures precision with time and may involve different analysts, equipment, and reagents. The certified value range, also known as "standard", is used to monitor the accuracy of the analysis and to validate the analytical method (32). The percentage of recovery is a crucial parameter for method validation. If the recovery percentage is small, the sample bias affects the method significantly and can lower its validity. Accuracy refers to the closeness of a measured value to a standard or known value (33). MDL is the minimum concentration of substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero (34).

Relative standard deviation

The relative standard deviation (RSD) is used to compare the uncertainty between different measurements of varying absolute magnitude. The RSD is calculated from the standard deviation (s) and is commonly expressed as parts per thousand (ppt) or percentage (%). A high RSD indicates that the values are widely distributed around the average value, whereas a low RSD means that the values are close to the average. The formula for RSD is:

$$\text{RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Statistical analysis

One-way repeated measure analysis of variance (ANOVA) was used in this study to identify the differences between three digestion methods. The Shapiro-Wilk statistic was used to test the assumption of normality, and Mauchly's test was used to examine the sphericity. In addition, because groups have the same sample size, a Post-hoc Bonferroni test was performed to identify significant differences among methods.

Results

Trace elements between digestion methods

For hair samples, significant differences in the Se, Mn, Mg, and Zn level were found between methods (Table 2). In this study, significant differences in the level of elements were found between wet acid digestion using only HNO_3 and wet acid digestion using HNO_3 and H_2O_2. For nail samples, only Se showed significant difference between all methods and Cu showed no significant difference between methods (Table 3). Other elements demonstrated significant difference for only one method: Mn is significantly higher by wet acid digestion using HNO_3, and Mg and Zn are significantly lower by dry ashing.

Precision

For hair samples, the within-run precision of Cu, Mn, Mg, and Zn for all methods was lower than 5% RSD (Table 4). However, the within-run precision of Se for all methods was higher than 10% RSD, with the lowest precision obtained for the dry ash method (177%). For all elements, the between-run precision of the three methods showed poorer results than the within-run precision. For the wet acid digestion method using HNO_3, only Mn and Mg showed an RSD better than 10%. Cu, Se, and Zn showed 17.13%, 14.3%, and 13.12% RSD, respectively. The between-run precision of wet acid digestion using HNO_3 and H_2O_2 showed quite similar results, with RSD values for Cu, Se, and Zn ranging between 11.03% and 17.96%. For nail samples, the within- and between-run precisions showed quite similar results (Table 5).

Accuracy

The accuracy of the digestion methods is assessed using CRM of human hair developed by the IAEA, (IAEA-086). For wet acid digestion using HNO_3, only the Se value agrees well with the certified range (Table 6). Wet acid digestion using HNO_3 and H_2O_2 showed similar results for Se, Mn, and Mg, which were in agreement with the certified values. Despite their poor precision

Table 2: Level of trace element in subjects' hair according to digestion method (µg /g)

Element	Digestion Methods Mean (SD); n= 20			*P* values
	Wet Acid (HNO_3)	Wet Acid (HNO_3 & H_2O_2)	Dry Ash	
Cu	10.673 (1.84)	10.822 (1.09)	8.014 (1.31)[a,b]	0.009
Se	0.312 (0.05)	0.380 (0.05)[a]	0.147 (0.03)[a,b]	< 0.001
Mn	3.941 (0.91)	3.319 (0.65)[a]	9.550 (2.00)[a,b]	< 0.001
Mg	49.67 (3.76)	45.09 (5.07)[a]	38.10 (6.05)[a,b]	< 0.001
Zn	294.45 (49.93)	275.12 (44.74)[a]	240.96 (23.69)[a,b]	< 0.001

One-way repeated measure ANOVA test.
[a] Significantly different ($P < 0.05$) compared to wet acid (HNO_3) method.
[b] Significantly different ($P < 0.05$) compared to wet acid (HNO_3 & H_2O_2) method

Table 3: Level of trace elements in subject's nail according to digestion methods (µg/g)

Element	Digestion Methods Mean (SD); n= 20			*P* values
	Wet Acid (HNO_3)	Wet Acid (HNO_3 & H_2O_2)	Dry Ash	
Cu	5.714 (1.22)	5.234 (1.13)	4.985 (0.86)	0.100
Se	0.688 (0.17)	0.501 (0.06)[a]	0.104 (0.02)[a,b]	< 0.001
Mn	1.555 (0.35)	0.963 (0.40)[a]	1.243 (0.16)[a]	< 0.001
Mg	66.90 (16.81)	61.53 (10.35)	53.76 (7.52)[a,b]	0.005
Zn	101.28 (16.74)	105.17 (19.87)	86.93 (14.47)[a,b]	0.004

One-way repeated measure ANOVA test.
[a] Significantly different ($P < 0.05$) compared to wet acid (HNO_3) method.
[b] Significantly different ($P < 0.05$) compared to wet acid (HNO_3 & H_2O_2) method.

for Se, these two methods showed accurate results for the CRM sample.

Percentage of recovery

Good recovery was observed for Se in the wet acid digestion method using HNO_3 and for Mn and Mg in the wet acid digestion using HNO_3 and H_2O_2 and in the dry ash method (Table 7). The level of Se obtained by wet acid digestion using HNO_3 and H_2O_2 agrees well with the certified value, but showed sub-par recovery. High recovery, beyond the acceptable limit, was obtained for Cu, Mn, Mg, and Zn in the wet acid digestion using HNO_3 and for Cu and Zn in the wet acid digestion using HNO_3 and H_2O_2. In agreement with the accuracy results, the dry ash method gave poor elemental recovery (only 5% of Se recovery).

Table 4: Relative standard deviation (RSD) of trace elements in subject's hair according to digestion methods (%)

Element	Digestion Methods (n = 20)					
	Wet Acid (HNO_3)		Wet Acid (HNO_3 & H_2O_2)		Dry Ash	
	Within run	Between run	Within run	Between run	Within run	Between run
Cu	2.09	17.13	1.32	17.96	1.30	20.91
Se	15.63	14.53	15.92	11.03	177.15	46.67
Mn	5.07	6.48	1.19	7.57	1.62	102.76
Mg	1.79	3.19	1.88	6.14	1.50	26.91
Zn	2.38	13.21	1.43	13.40	1.65	13.56

Table 5: Relative standard deviation (RSD) of trace elements in subject's nail according to digestion methods (%)

Element	Digestion Methods (n = 20)					
	Wet Acid (HNO_3)		Wet Acid (HNO_3 & H_2O_2)		Dry Ash	
	Within run	Between run	Within run	Between run	Within run	Between run
Cu	1.74	8.67	1.68	3.73	1.58	20.97
Se	20.07	5.44	28.71	10.24	66.14	61.65
Mn	1.73	6.90	1.88	7.57	1.36	18.96
Mg	1.59	6.79	1.56	10.64	1.93	13.00
Zn	1.62	6.91	2.05	11.52	1.50	10.34

Table 6: Level of trace elements for IAEA-086 Human Hair according to digestion methods (µg/g)

Element	Digestion Methods Mean (SD); n=3			Certified Value Range
	Wet Acid (HNO_3)	Wet Acid (HNO_3 & H_2O_2)	Dry Ash	
Cu	25.5 (0.05)	24.79 (0.02)	14.43 (0.03)	17.6 (1.89)
Se	1.22 (0.03)*	0.84 (0.03)*	0.05 (0.04)	1.00 (0.32)
Mn	18.77 (0.06)	10.59 (1.3)*	10.49 (0.01)*	9.6 (1.56)
Mg	278.5 (1.24)	171.65 (0.7)*	157.20 (0.53)*	177 (35.3)
Zn	240.85 (0.36)	221.46 (0.45)	119.45 (0.33)	167 (17.5)

* Wet acid digestion using HNO3 and H2O2 method showed similar result with Se, Mn and Mg were in agreement with the certified ranges.

MDL

In this study, the MDL for the three procedures tested were compared with the MDL values previously reported for the microwave digestion method (Table 8). For all elements, the MDL of the three methods are equal to or higher than the published MDL values. Only for Se, the MDL of the dry ash method was higher than the published value.

Physical comparison between the digestion methods

Besides the study of the analytical performances of the three methods, physical

Table 7: Percentage of recovery of IAEA-086 Human Hair according to digestion methods (%)

Element	Digestion Methods		
	Wet Acid (HNO_3)	Wet Acid (HNO_3 & H_2O_2)	Dry Ash
Cu	144	141	82
Se	110*	85	5
Mn	195	110*	109*
Mg	157	97*	90
Zn	144	132	72

Percentage of Recovery = $\frac{\text{The Analysed Concentration of Reference Material}}{\text{The Certified Concentration of Reference Material}} \times 100$

*Sample Shows Good Percentage of Recovery.

Table 8: Method detection limit (MDL) of trace elements according to digestion methods (µg/g)

Element	Digestion Methods			
	Wet Acid (HNO_3)	Wet Acid (HNO_3 & H_2O_2)	Dry Ash	Rodushkin & Axelsson
Cu	1.3	0.4	0.5	0.034
Se	0.1	0.02*	0.005	0.025
Mn	0.07	0.07	0.1	0.06
Mg	0.25*	0.2*	0.2*	0.22
Zn	0.1	0.1	1.5	0.07

*Method which have same or lower MDL than the previous study.
Source : Rodushkin I, Axelsson MD. Sci Total Environ 2000; 250: 83-100.

Table 9: Physical comparison of the digestion methods

Parameters	Wet Acid (HNO_3)	Wet Acid (HNO_3 & H_2O_2)	Dry Ash
Estimation of reagent cost per sample	< MYR 0.50	< MYR 1.00	< MYR 0.50
Turnaround time per batch	One day	Two days	One day
Risk of contamination	High	Low	High
Element losses	Volatile elements is at risk of loss	Low risk of losses	Volatile elements is at risk of loss
Supervision	Requires supervision	Does not requires supervision	Requires supervision
Safety risks	Hazardous fumes, corrosive	Hazardous fumes, corrosive	Hazardous fumes, corrosive
Instrument requirement	Requires block digester	Requires drying oven	Requires electrical furnace

Abbreviation: MYR= Malaysia Ringgit.

comparisons were made, based on various parameters (Table 9).

Discussion

For hair samples, significant differences in the Se, Mn, Mg, and Zn level were found between methods. Demirel et al. (21) and Aydin (20) reported slight differences in the content of elements between dry ashing and wet acid digestion. However, studies conducted by Ming and Bing (13) showed no statistical differences between dry ash and wet acid digestion in terms of accuracy. The significant differences in element contents may be attributed to the various disadvantages of these methods, such as contamination during sample processing, mainly due to open system procedures. Trace metals are mobile and can come from soil, air, industrial processes, and transportation (21). According to Hoenig (22), for these digestion methods, contamination can derive from the reagents and materials used, from ambient air, and from the decomposition of products.

Frequently observed contaminations are due to the systematic or random introduction of non-negligible amounts of analyte at different stages of the analysis. In addition, trace elements can be lost by adsorption to the vessel walls or by volatilisation (23). The dry ash method is known to cause loss of elements, namely As, Hg, and Se, by volatilisation because of the high temperatures used (22). This explains the significantly lower level of Se found with the dry ash method as compared with that obtained by other methods.

In this study, significant differences in the level of elements were found between wet acid digestion using only HNO_3 and wet acid digestion using HNO_3 and H_2O_2. According to Clegg et al. (24), the use HNO_3 is more effective in terms of recovery as compared with the combination of HNO_3 and H_2O_2. On the other hand, Aydin (20) claimed that the combination of HNO_3 and H_2O_2 afforded better results and dissolution. In the present study, we also observed poor solubility when HNO_3 and H_2O_2 are used separately. This confirms that these two procedures can produce different results, as found herein.

Nail samples showed more consistent results between the methods as compared with hair samples. External contamination, such as dust and oil (17), on the hair could interfere with the interpretation of the results, complicating the determination of endogenous element levels. For this reason, a hair washing procedure is applied. However, to date, no standard hair washing procedure has been established, and an optimal treatment cannot be adopted (25). Because trace elements have different binding capabilities, the identification of a washing procedure for a large range of elements is quite difficult (26). Further studies on this complex subject are beyond the scope of this research.

Both within-run and between-run precisions were measured. Within-run precision indicates the variation of results on a given day and consisted of digesting three separate aliquots of the 20 samples of hair and nails. The between-run precision is measured by digesting and analysing the same 20 samples on three non-consecutive days, in order to evaluate the day-to-day variations. The poor RSD of Se may be attributed to its low level in hair and nails as compared with that of the other elements; thus, an error is likely to occur, probably due to loss by vaporisation during the digestion process (22), which is consistent with the worse RSD values found for the dry ash method.

For all elements, the between-run precision of the three methods showed poorer results than the within-run precision. For the wet acid digestion method using HNO_3, only Mn and Mg showed an RSD better than 10%. According to Twyman (23), during wet acid digestion, the open systems are prone to contamination, which may occur from the environment, from the reagents during storage, and from impurities present in the reagents (22). In the present study, in order to minimise possible contamination, various measures have been taken, such as acid washing of all equipment involved and performing the analysis in a dust-free space. However, meticulous efforts, such as performing the test in a clean room, as suggested by Ming and Bing (13), and purifying the reagents used (22), could not be made in this work. Thus, contamination is inevitable. Moreover, this method is often dependent on the skill of the operator, because it is difficult to standardise and reproduce; hence, human error is also likely (12). The between-run precision of wet acid digestion using HNO_3 and H_2O_2 showed quite similar results. Although this method is a closed system, contamination during pipetting, from the tubes, from the reagent storage bottles, and from the reagents used is still possible. It is difficult to identify the origin of the error and the element affected. This method is also dependent on the operator's skill for reproducibility (12).

The dry ash method showed the highest day-to-day variation for all elements. This is due to its open digestion system and to the long heating processes, which can lead to contamination from the environment, the tubes, the crucible bowl,

and during transfers. The high temperature used will also inevitably cause volatilisation losses of elements such as Se (22) and Cu (27). In addition, Friel and Ngyuen reported that Mn and Zn can be adsorbed on the crucible walls (27). For nail samples, the within- and between-run precisions showed quite similar results. Briefly, both wet acid digestion using HNO_3 and wet acid digestion using HNO_3 and H_2O_2 showed good within- and between-run precision for Mn and Mg. Among the three methods, wet acid digestion using HNO_3 and H_2O_2 showed the best RSD range within- and between-run.

The accuracy of the digestion methods is assessed using CRM of human hair. For wet acid digestion using HNO_3, only the Se value agrees well with the certified range. In this method, samples may have been susceptible to contamination with other elements, but not with Se because it is not prevalent in the environment (25). Wet acid digestion using HNO_3 and H_2O_2 showed similar results for Se, Mn, and Mg. Despite their poor precision for Se, these two methods showed accurate results for the CRM sample. This may possibly be the result of a random, human or instrumental, error. Moreover, wet acid digestion is often difficult to standardise and reproduce; in addition, because the concentration of Se is low as compared with that of other elements, it is prone to imprecision (12). This suggests that the temperature used in these methods does not cause volatilisation of Se.

Wet acid digestion using HNO_3 and H_2O_2 showed great accuracy and precision for Mn and Mg, indicating no random and systematic error affecting their measurement. With this method, Cu and Zn values were over the certified range, which suggests contamination. Dry ashing gave Mn and Mg values that agree well with the certified ranges, but with poor precision. This can be caused by human error or random contaminants. Poor accuracy was observed for Cu, Se, and Zn, with values lower than the certified ranges. Presumably, and according to Hoenig (22), the temperature used in the dry ash method causes loss of some elements, such as Se (22) and Cu (27), by volatilisation. In addition, Friel and Ngyuen reported that Zn may be retained on the crucible walls during the ashing process (27).

The percentage of recovery is the amount of element left or recovered from the original sample after the digestion process, and is therefore related to the accuracy. In agreement with the accuracy results, good recovery was observed for Se in the wet acid digestion method using HNO_3 and for Mn and Mg in the wet acid digestion using HNO_3 and H_2O_2 and in the dry ash method. High recovery, beyond the acceptable limit, in the wet acid digestion using HNO_3 and in the wet acid digestion using HNO_3 and H_2O_2 is often associated with contamination (27). Presumably, the temperature used for dry ashing caused the loss of this element by volatilisation.

MDL is the smallest amount or concentration of a particular substance that can be reliably detected in a given type of sample or medium by a specific method (28). Moreover, a high MDL value may indicate the presence of impurities and contaminants. It is calculated as three times the standard deviation for digestion blanks and expressed as equivalent concentrations in the samples (29). Microwave digestion is the most suitable method for standardisation (9), because of the simple, effective, and clean sample preparation process (20).

The results indicated that, in general, among the three methods, wet acid digestion using HNO_3 and H_2O_2 requires the cleanest sample preparation technique. The dry ash method showed a higher MDL for Cu, Mn, and Zn as compared with the other methods. The wet acid digestion using HNO_3 yielded a higher MDL for Cu and Se than the wet acid digestion using HNO_3 and H_2O_2. Because the wet acid digestion using HNO_3 and the dry ash method involve open system processes, they are more subjected to contamination as compared with wet acid digestion using HNO_3 and H_2O_2 (22). Moreover, in both methods, transferring procedures may increase the possibility of contamination. Nevertheless, for all three methods, the MDL are adequate for the determination of all elements at the concentrations used.

The use of HNO_3 is associated with the risk of hazards, toxic fumes, and corrosion; thus, precautions must be taken when performing the three methods, such as wearing personal protective equipment (PPE) (e.g. mask, lab coat, and gloves) and working in a well-functioning fume hood. The three digestion procedures required different equipment, such as block digester, drying oven, and electric furnace. The goal of this study is not to determine the optimal approach, but rather to discuss the factors to be considered in selecting the most appropriate digestion method for a specific research.

Conclusion

Wet acid digestion using HNO_3 and H_2O_2 was the most reliable digestion method in this study, as compared with wet acid digestion using HNO_3

and dry ash method. This conclusion is based on the precision, accuracy, percentage of recovery, and MDL of Cu, Se, Mn, Mg, and Zn.

In addition, the method showed low risk of contamination and loss of volatile species, and it does not require supervision. However, results can be considered valid only when the element level is both precise and accurate, which is observed only for Mn and Mg obtained through wet acid digestion using HNO_3 and H_2O_2, because of the closed system conditions used. Improvements to the procedure can be made in the future to achieve better results.

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Conflict of Interest

None.

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Authors' Contributions

Conception and design, critical revision of the article for important intellectual content, final approval of the article: II, JM
Analysis and interpretation of the data, provision of study materials or patients, collection and assembly of data: FDR
Drafting of the article: FDR, MFMI
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Administrative, technical, or logistic support: MFMI

Correspondence

Dr Ismarulyusda Ishak
BSc Biomedicine (UKM), PhD
Faculty of Health Sciences,
School of Diagnostic and Applied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur, Malaysia
Tel: +603-9289 7615
Fax: +603-2692 9032
Email: ismarul@fsk.ukm.my

References

1. Cai Y. Determination Of Select Trace Elements In Hair Of College Students In Jinzhou, China. *Biol Trace Elem Res*. 2011;**144(1–3)**:469–474. doi: 10.1016/j.ajhg.2013.04.05.
2. Bornhorst JA, McMillin GA. Trace And Toxic Elemental Testing In The Clinical Laboratory. *Lab Med*. 2006;**37(11)**:690–695. doi: 10.1309/XN6BLHL1Q14VXG9M.
3. He K. Trace Element In Nails As Biomarkers In Clinical Research. *Eur J Clin Invest*. 2011;**41(1):**98–102. doi: 10.1111/j.1365-2362.2010.02373.x.
4. Laker M. On Determining Trace Element Levels In Man: The Uses Of Blood And Hair. *The Lancet*. 1982;**320(8292)**:260–262. doi: org/10.1016/s0140-6736(82)90336-1.
5. Rao KS, Balaji T, Rao TS, Babu Y, Naidu GRK. Determination Of Iron Cobalt, Nickel, Manganese, Zinc, Copper, Cadmium And Lead In Human Hair By Inductively Coupled Plasma-Atomic Emission Spectrometry. *Spectrochim Acta Part B At Spectrosc*. 2002;**57(8)**:1333–1338. doi: org/10.1016/s0584-8547(02)00045-9.
6. Rodushkin I, Axelsson MD. Application Of Double Focusing Sector Field ICP-MS For Multielemental Characterization Of Human Hair And Nails. *Sci Total Environ*. 2000;**250(1)**:83–100. doi: org/10.1016/s0048-9697(00)00369-7.
7. Ogboko B. Trace Element Indices In Hair And Saliva Of School Children. *J Basic Appl Sci Res*. 2010;**1(3)**:169–177. doi: org/10.4314/jbi.v6i2.65837.
8. Jung RS, Yang SR, Han JK, Kang GW, Lee GH. Determination Of Lead, Cadmium, And Chromium In Hair Optimized By Simplex Method Using Electrothermal Vaporization-Inductively Coupled Plasma Mass Spectrometry. *Anal Sci*. 2001;**17(0)**:i999–i1002. doi: org/10.1039/a800485d.
9. Bass DA, Hickok D, Quig D, Urek K. Trace Element Analysis In Hair: Factors Determining Accuracy, Precision, And Reliability. *Altern Med Rev*. 2001;**6(5)**:472–480. doi: org/10.1007/bf02783970.
10. Goulle JP, Saussereau E, Mahieu L, Bouige D, Groenwont S, Guerbet M, et al. Application Of Inductively Coupled Plasma Mass Spectrometry Multielement Analysis In Fingernail And Toenail As A Biomarker Of Metal Exposure. *J Anal Toxicol*. 2009;**33(2)**:92–98. doi: org/10.1093/jat/33.2.92.
11. Montaser A, Golightly DW. *Inductively Coupled Plasmas In Analytical Atomic Spectrometry*. New York: VCH, 1987. doi: org/10.1039/ja9880300965.
12. Puchyr RF, Bass DA, Gajewski R, Calvin M, Marquardt W, Urek K, et al. Preparation Of Hair For Measurement Of Elements By Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). *Biol Trace Elem Res*. 1998;**62(3):**167–182. doi: org/10.1007/bf02783969.

13. Ming Y, Bing L. Determination Of Rare Earth Elements In Human Hair And Wheat Flour Reference Materials By Inductively Coupled Plasma Mass Spectrometry With Dry Ashing And Microwave Digestion. *Spectrochim Acta Part B At Spectrosc*. 1998;**53(10)**:1447–1454. doi: org/10.1016/s0584–8547(98)00159-1.

14. Sukumar A. Human Nails As A Biomarker Of Element Exposure. *Rev Environ Contam Toxicol*. 2006;**185**:141–177. doi: org/10.1007/0-387-30638-2_5.

15. da Silva JB, Borges DL, da Veiga MA, Curtius AJ, Welz B. Determination Of Cadmium In Biological Samples Solubilized With Tetramethylammonium Hydroxide By Electrothermal Atomic Absorption Spectrometry, Using Ruthenium As Permanent Modifier. *Talanta*. 2003;**60(5)**:977–982. doi: org/10.1016/s0039-9140(03)00182-6.

16. Batista BL, Rodrigues JL, Nunes JA, Tormen L, Curtius AJ, Barbosa F. Simultaneous Determination Of Cd, Cu, Mn, Ni, Pb, And Zn In Nails Samples By Inductively Coupled Plasma Mass Spectrometry (ICP-MS) After TMAH At Room Temperature. *Talanta*. 2008;**76(3)**:575–579. doi: org/10.1016/j.talanta.2008.03.046.

17. Ryabukhin YS. Activation Analysis Of Hair As An Indicator Of Contamination Of Man By Environmental Trace Element Pollutants. IAEA Report IAEA/RL/50. *Vienna*. 1978. doi: org/10.1016/b978-0-08-021948-6.50083-2.

18. Miekeley N, Dias Carneiro MT, da Silveira CL. How Reliable Are Human Hair Reference Intervals For Trace Elements? *Sci Total Environ*. 1998;**218(1)**:9–17. doi: org/10.1016/s0048-9697(98)00185-5.

19. Wongwit W, Kaewkungwal J, Chantachum Y, Visesmanee V. Comparison Of Biological Specimens For Managanese Determination Among Highly Exposed Welders. *Southeast Asian J Trop Med Public Health*. 2004;**35(3)**:764–769.

20. Aydin I. Comparison Of Dry, Wet And Microwave Digestion Procedures For The Determination Of Chemical Elements In Wool Samples In Turkey Using ICP-OES Technique. *Microchem J*. 2008;**90(1)**:82–87. doi: org/10.1016/j.microc.2008.03.011.

21. Demirel S, Tuzen M, Saracoglu S, Soylak M. Evaluation Of Various Digestion Procedures For Trace Element Contents Of Some Food Materials. *J Haz Mat*. 2008;**152(3)**:1020–1026. doi: org/10.1016/j.jhazmat.2007.07.077.

22. Hoenig M. Preparation Steps In Environmental Trace Element Analysis – Facts And Traps. *Talanta*. 2001;**54(6)**:1021–1038. doi: org/10.1016/s0039-9140(01)00329-0.

23. Twyman RM. *Sample Dissolution for Elemental Analysis/Wet Digestion*. Encyclopedia of Analytical Chemistry. 2nd Ed. Oxford: Elsevier; 2005. doi: org/10.1016/b0-12-369397-7/00539-2.

24. Clegg M, Keen CL, Lonnerdal B, Hurley HS. Influence Of Ashing Techniques On The Analysis Of Trace Elements In Animal Tissue. *Biol Trace Elem Res*. 1981;**3(2)**:107–115. doi: org/10.1007/bf02990451.

25. Morton J, Carolan VA, Gardiner PHE. Removal Of Exogenously Bound Elements From Human Hair By Various Washing Procedures And Determination By Inductively Coupled Plasma Mass Spectrometry. *Anal Chim Acta*. 2002;**455(1)**:23–24. doi: org/10.1016/s0003-2670(01)01578-1.

26. Chyla MA, Zyrnicki W. Determination Of Metal Concentration In Animal Hair By The ICP Method. Comparison Of Various Washing Procedures. *Biol Trace Elem Res*. 2000;**75(1–3)**:187–194. doi: org/10.1385/bter:75:1–3:187.

27. Friel JK, Ngyuen CD. Dry And Wet Ashing Techniques Compared In Analyses For Zinc, Copper, Manganese, And Iron In Hair. *Clin Chem*. 1986;**32(5)**:739–742.

28. Curie LA. Detection: International Update, And Some Emerging Dilemmas Involving Calibration, The Blank, And Multiple Detection Decisions. *Chemometrics Intel Lab Sys*. 1997;**37(1)**:151–181. doi: org/10.1016/s0169-7439(97)00009-9.

29. Rodushkin I, Ruth T, Huhtasaari A. Comparison Of Two Digestion Methods For Elemental Determinations In Plant Material By ICP Techniques. *Anal Chim Acta*. 1999;**378(1)**:191–200. doi: org/10.1016/s0003-2670(98)00635-7.

30. Freiser H. *Concept Calculations in Analytical Chemistry*.Chapter 12. Boca Raton (FL):CRC Press; 1992. p. 203.

31. Douglas C. Evaluating Assay Precision. *Clin Biochem Rev*. 2008;**29(1)**:S23–S26.

32. Guimaraes EdF, do Rego EC, Cunha H, Rodrigues JM, Figueroa-Villar JD. Certified Reference Material For Traceability In Environmental Analysis: PAHs In Toluene. *J Braz Chem Soc*. 2014;**25(2)**:351–360. doi: org/10.5935/0103-5053.20130303.

33. Taverniers I, Loose MD, Bockstaele EV. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends Anal. Chem*. 2004:**23(8)**:535–552. doi: 10.1016/j.trac.2004.04.001.

34. Armbruster DA, Pry T. Limit of Blank, limit of Detection and Limit of Quantitation. *Clin Biochem Rev*. 2008;**29(Suppl 1)**:S49–S52. PMCID: PMC 255658.