


TECHNICAL NOTE

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Revisited digestion methods for trace element analysis in human hair

Woo-Jin Shin¹, Minkyong Jung¹, Jong-Sik Ryu^{1,2,3*} , Jihwan Hwang^{1,3} and Kwang-Sik Lee^{1,3}

Abstract

Background: The human hair is a potential material for assessing the exposure to environmental contaminants and tracing human mobility. Although various digestion methods have been proposed for determining trace elements in the human hair, there is no consensus about the best method among them yet.

Findings: We examined five different methods in order to determine the best method yielding the most accurate and precise data of trace elements in the human hair using two certified reference human hairs (IAEA085 and IAEA086) under controlled conditions (temperature, the volume of hydrogen peroxide (H₂O₂), and the number of digestion). Results showed that a high temperature of 160 °C yields better recovery rates than a low temperature of 70 °C. Furthermore, the additional input of H₂O₂ increases the recovery rate from 90 to 102%, and the two-time digestion also promotes the recovery rate.

Conclusions: This study suggests that although the combination of high temperature (160 °C), high volume of H₂O₂ (0.4 mL), and two-time digestion yields the most accurate and precise data of trace elements in the human hair, the digestion method should be carefully selected depending on the content of organometallic cation.

Keywords: Human hair, Trace element, Temperature, Hydrogen peroxide, Organometallic cation

Introduction

The human hair is mostly composed of keratin (50% C, 21% N, 17.5% H, 6.5% O, and 5% S) with a small amount of trace elements, such as Ca, Fe, K, Na, and Sr (Font et al. 2012). Because trace elements are incorporated into the hair through diet and environmental exposure (Dongarrà et al. 2011), the determination of them can allow us to infer environment where a human was exposed during his or her life. For example, farmer's hairs informed the prolonged exposure to pesticides and fertilizers (Hamid et al. 2017). Furthermore, Varrica et al. (2014) showed that hairs of children who were grown at the contaminated area by abandoned mines yielded high concentrations of heavy metals. For these reasons, the human hair has been commonly used in forensic science and archaeology (Kempson et al. 2010; Pragst et al. 2017).

In this context, the critical issue is to accurately and precisely determine trace elements in the human hair. Many studies have commonly used a digestion method using a mixture of ~0.8 mL of nitric acid (HNO₃) and 0.2 mL of hydrogen peroxide (H₂O₂) to measure trace elements in the human hair and nail (e.g., Luo et al. 2014; Ishak et al. 2015), while experimental conditions, such as temperature (*T*), digestion duration, and apparatus, are slightly different from each other. For example, hair samples were reacted with a mixture of 1 mL of HNO₃ and 0.1 mL of H₂O₂ in an ultrasonic cleaner at *T* = 75 °C during 60 min (Barbieri et al. 2011). Tipple et al. (2018) digested hair samples using a microwave digestion system at *T* = 200 °C (13.3 °C/min rate) during 30 min, while Font et al. (2012) used a mixture of 1.5 mL of HNO₃ and 0.5 mL of HCl at *T* = 110 °C for 24 h, and then a mixture of 0.1 mL of HNO₃ and 0.1 mL of H₂O₂ at *T* = 105 °C for 10 min. Nonetheless, there is no standardized digestion method yet until now. Here, we examined five different digestion methods using two certified hair standards (IAEA085 and IAEA086) and compared them regarding the

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recovery rates relative to certified values. This study will provide a guideline for the human hair digestion.

Materials and methods

Sample

Two certified hair standards (IAEA085 and IAEA086; human hair) were used in this study, of which the former represents hair with an elevated level of methylmercury (MeHg^+ ; CH_3Hg^+), while the latter is representative of the endogenous level for MeHg^+ . The samples were cryogenically homogenized using the stainless steel “CryoPalla” mill at the KFA-Jülich Specimen Bank facility, until approximately 70% of the samples have a particle size below $71\ \mu\text{m}$. Then, the samples were γ -sterilized to ensure long-term stability of the material by inhibiting microbial action (IAEA, 2019a and 2019b).

Pre-cleaning

In order to remove possible contaminants, samples were cleaned using a 0.5% Triton X-100 (Sigma Aldrich), washed several times using a ACS grade acetone (Honeywell B & J), and finally washed three times using a MilliQ water following the procedures in Borella et al. (1996). Then, the pre-cleaned samples were completely dried in an oven at $T = 60\ ^\circ\text{C}$.

Digestion

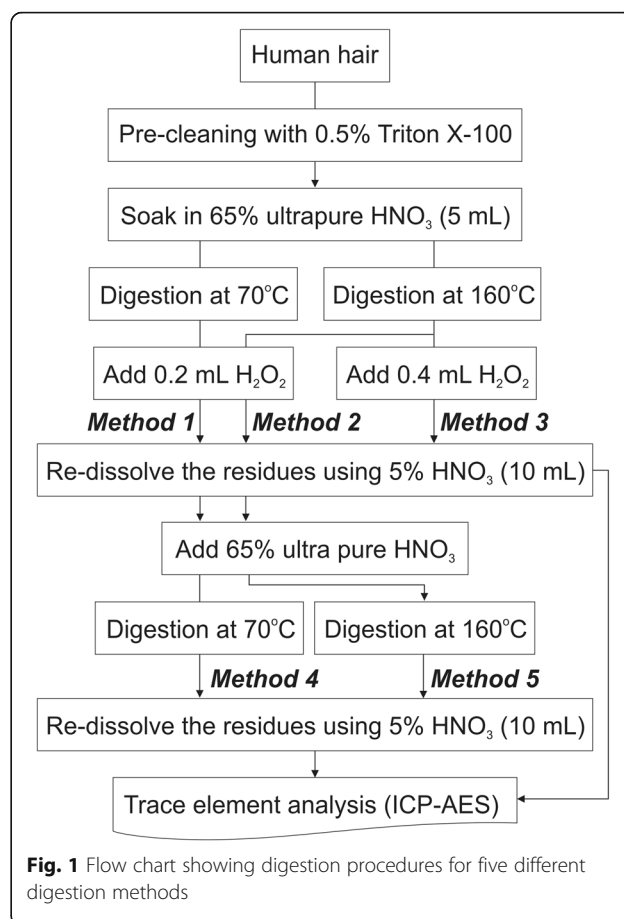
Pre-cleaned hair samples were digested using five different methods, in which the volume of reagent, reaction temperature, and the number of digestions were controlled (Fig. 1). Furthermore, both IAEA085 and IAEA086 samples were prepared in duplicate using method 3 in order to quantify analytical uncertainty.

First digestion

About 0.2 g of pre-cleaned hair samples were put in 60 mL Teflon vessels, and then, 5 mL of ultrapure HNO_3 was added. One sample was heated on a hot plate at $T = 70\ ^\circ\text{C}$ overnight (method 1), and the others were at $T = 160\ ^\circ\text{C}$ (methods 2 and 3). In order to investigate the effect of H_2O_2 on recovery rates, either 0.2 mL or 0.4 mL of H_2O_2 was added to the samples (methods 1 and 2, and method 3, respectively) and reacted for 30 min. The samples were dried and re-dissolved in 10 mL of 5% HNO_3 .

Second digestion

Two aliquots from methods 1 and 2 were dried and then additionally reacted with 10 mL of ultrapure HNO_3 in Teflon vessels. Each sample was digested on a hot plate at $T = 70\ ^\circ\text{C}$ (method 4) and $160\ ^\circ\text{C}$ (method 5) for 8 h. Then, the samples were dried and re-dissolved in 10 mL of 5% HNO_3 .



Elemental analyses

Concentrations of trace elements (Ca, Cu, Mg, Mn, Sr, Zn) were measured using a PerkinElmer Optima 8300 ICP-AES at the Korea Basic Science Institute (KBSI). Repeated analyses of two certified standards (Trace Metals in Drinking Water, TMDW; and river water for trace metals and other constituents, SLRS-6) yielded analytical accuracy better than $\pm 5\%$. Furthermore, the analytical uncertainty for both IAEA085 and IAEA086 samples was less than 4% for all elements, ranging from 0.1 to 0.9% and from 0.8 to 3.6%, respectively (Table 1).

Results and discussion

Table 1 presents measured and certified values of trace elements (Ca, Cu, Mg, Mn, Sr, Zn) for IAEA085 and IAEA086.

Effect of temperature: methods 1 versus 2

Most of the samples prepared by first digestion showed the recovery rate of approximately 73 to 96% for all trace elements (Table 1; Fig. 2a). Between $T = 70$ and $160\ ^\circ\text{C}$, a high temperature yielded much better recovery rates than a low temperature in both IAEA085 and IAEA086 samples (see Table 1; IAEA085-70 and IAEA086-70)

Table 1 Trace elements in two certified reference human hairs (IAEA085 and IAEA086)

Sample	Method	Temperature (°C)	Volume of H ₂ O ₂ (mL)	Number of digestion	Ca (µg/g)	Cu (µg/g)	Mg (µg/g)	Mn (µg/g)	Sr (µg/g)	Zn (µg/g)
IAEA085 ^a					929	16.8	140	8.8	–	163
IAEA085-70	Method 1	70	0.2	1	815 (88)	14.6 (87)	123 (88)	6.4 (73)	7.6	137 (84)
IAEA085-160	Method 2	160	0.2	1	818 (88)	14.4 (86)	127 (91)	6.9 (79)	7.5	127 (78)
IAEA085-160HP	Method 3	160	0.4	1	845 (91)	–	127 (91)	8.6 (98)	7.5	157 (96)
IAEA085-160HP-replicate					842 (91)	15.3 (91)	126 (90)	8.6 (98)	7.4	158 (97)
IAEA085-70S	Method 4	70	0.2	2	805 (87)	14.8 (88)	121 (87)	6.1 (69)	7.9	173 (106)
IAEA085-160S	Method 5	160	0.2	2	947 (102)	17.3 (103)	141 (101)	8.6 (98)	9.1	179 (110)
IAEA086 ^a					1120	17.6	177	9.6	8.4	167
IAEA086-70	Method 1	70	0.2	1	884 (79)	15.2 (86)	139 (79)	8.4 (87)	7.7 (92)	158 (95)
IAEA086-160	Method 2	160	0.2	1	940 (84)	15.5 (88)	142 (80)	8.6 (89)	7.8 (93)	161 (96)
IAEA086-160HP	Method 3	160	0.4	1	1106 (99)	–	174 (98)	9.7 (101)	8.8 (105)	156 (94)
IAEA085-160HP-replicate					1067 (95)	15.8 (90)	170 (96)	9.8 (102)	8.6 (102)	158 (95)
IAEA086-70S	Method 4	70	0.2	2	939 (84)	15.6 (88)	142 (80)	8.6 (90)	7.9 (95)	159 (96)
IAEA086-160S	Method 5	160	0.2	2	957 (85)	16.7 (95)	148 (84)	8.8 (91)	8.3 (99)	165 (99)

The numbers in the parenthesis represent the recovery rate (%) calculated by dividing measured value by certified value

^aData from GeoReM (<http://georem.mpch-mainz.gwdg.de>)

although both methods display much less than 100% of recovery rate. For example, the recovery rates of Ca (84%), Cu (88%), Mg (80%), Mn (89%), and Zn (96%) in IAEA086-160 were slightly higher than those in IAEA086-70: Ca (79%), Cu (86%), Mg (79%), Mn (87%), and Zn (95%). During the experiment, after digestion with HNO₃ at $T = 70^\circ\text{C}$, the final solution showed dark pale yellow, whereas the high temperature showed bright pale yellow. This result indicated that a high temperature allowed organic materials in hair samples to efficiently decomposed, and therefore yielded higher recovery rates.

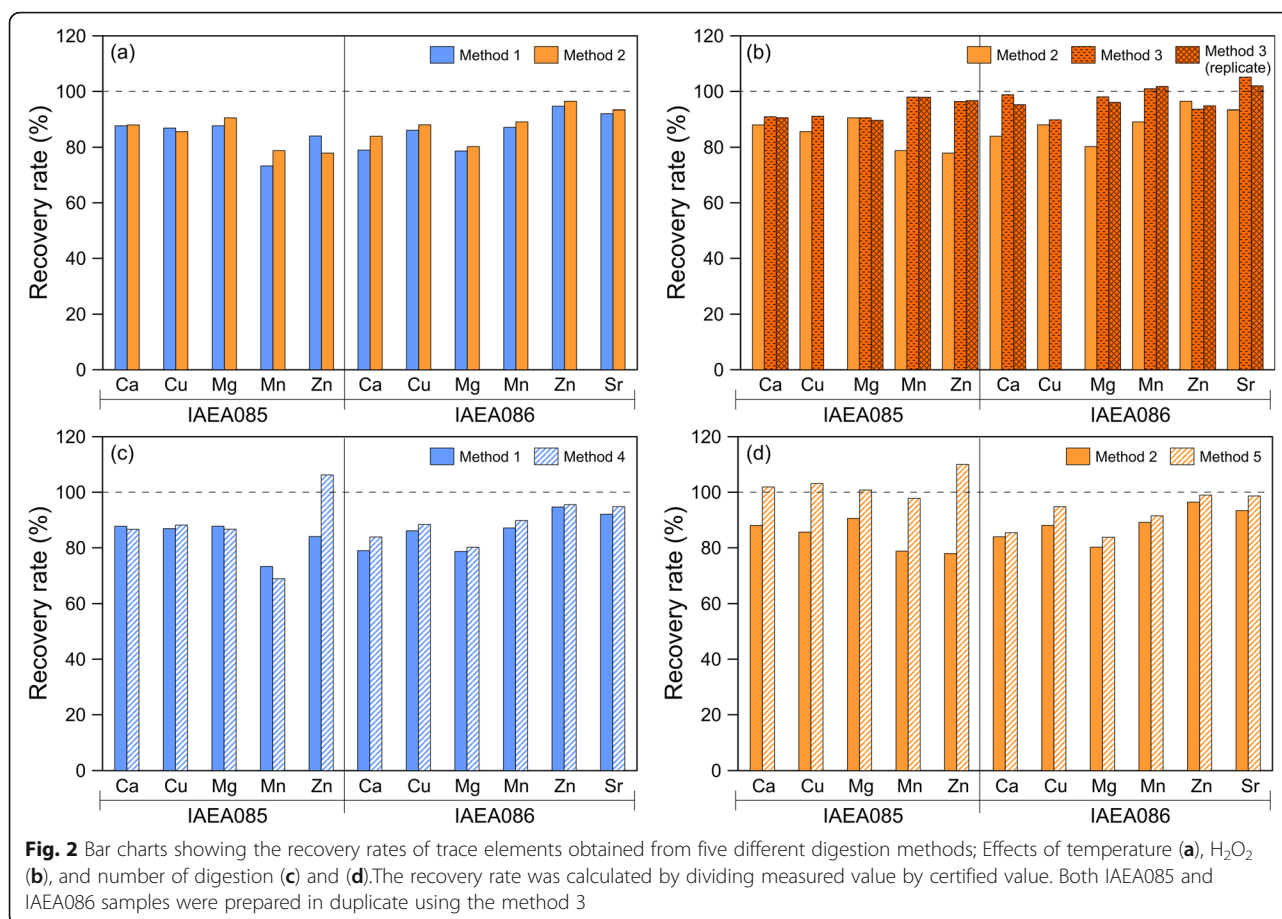
Effect of H₂O₂: methods 2 versus 3

In order to promote the recovery rate and examine the effect of H₂O₂, we added different volume of H₂O₂ during the digestion at $T = 160^\circ\text{C}$. The result showed that a high volume of H₂O₂ (0.4 mL) enhanced the decomposition of organic materials yielding high recovery rate compared to a low volume of H₂O₂ (0.2 mL). For example, IAEA085-160 method yielded the recovery rates of 88% for Ca, 86% for Cu, 91% for Mg, 79% for Mn, and 78% for Zn, while IAEA085-160HP showed better recovery rates of > 90% in all trace elements: 91% for Ca, 91% for Cu, 90% for Mg, 98% for Mn, and 97% for Zn (Fig. 2b). The similar results were observed in the IAEA086 sample (IAEA086-160 and IAEA086-160HP; Table 1), but the effect of H₂O₂ is much clearer in the IAEA086 sample. It could be due to the difference of MeHg⁺ content in the IAEA085 (22.9 mg/kg; IAEA, 2019a) and IAEA086 (0.258 mg/kg; IAEA, 2019b) samples, causing inefficient decomposition of high

organometallic cation in IAEA085 although the same volume of H₂O₂ was added. The amount of samples used in this experiment is much more than the recommended sample size for inorganic analyses (> 10 mg) (IAEA, 2019a and 2019b), deteriorating complete decomposition of organic compounds, especially for IAEA085 with higher MeHg⁺ content (22.9 mg/kg). Organic compounds in hair samples may be incompletely oxidized during the digestion using even a microwave oven, causing inaccurate analytical result (Novozamsky et al. 1995; Almeida et al. 1999). In short, our results indicated that the more volume of H₂O₂, the better recovery rate of trace elements in hair samples.

Effect of number of digestion: methods 1 versus 4, and 2 versus 5

Two digested samples, IAEA085 (or 086)-70 and IAEA085 (or 086)-160, were digested again at $T = 70^\circ\text{C}$ and 160°C (IAEA085 (or 086)-70S and IAEA085 (or 086)-160S, respectively) in order to examine the effect of the number of digestion on trace element analysis. As shown above, the results showed that high temperature yielded better recovery rates for all analyzed elements. Furthermore, two-time digestion greatly enhanced the recovery rate of Zn (up to 22%) in IAEA085-70S (method 4) compared to IAEA085-70 (method 1) (Table 1) but little differences in other elements (Fig. 2c). On the contrary, IAEA085 (or 086)-160S (method 5) showed much better recovery rates for all elements than IAEA085 (or 086)-160 (method 2), yielding the recovery rates of most element range between 91 and 103% (Fig. 2d). The effect of the number of digestion on the recovery rate is much



remarkable in IAEA085, showing the recovery rates of 102% for Ca, 103% for Cu, 101% for Mg, 98% for Mn, and 110% for Zn (Fig. 2c, d; Table 1). The result indicated that samples containing high content of organometallic cation (IAEA085) should be digested twice at higher temperature ($T = 160^{\circ}\text{C}$) in order to efficiently decompose it. Overall, this study indicates that the combination of high temperature ($T = 160^{\circ}\text{C}$) and much volume of H₂O₂ (0.4 mL) is the best method (method 3) for digesting human hair having low content of organometallic cation (IAEA086), while that of high temperature ($T = 160^{\circ}\text{C}$), H₂O₂ (0.2 mL), and two-time digestion is the best one (method 5) for human hair containing high content of organometallic cation (IAEA085).

Conclusions

The best digestion methods for trace element analysis in the human hair were examined using two certified reference human hairs, IAEA085 and IAEA086, under controlled experimental conditions. The results showed that human hair containing high content of organometallic cation (MeHg⁺), IAEA085, was completely digested using the combination of high temperature ($T = 160^{\circ}\text{C}$), H₂O₂ (0.2 mL), and two-time digestion, while that with

low content of organometallic cation (IAEA086) using the combination of high temperature ($T = 160^{\circ}\text{C}$) and much volume of H₂O₂ (0.4 mL). This study highlights that the combination of high temperature and several times digestions allows to better determine trace elements in the human hair containing a high content of organic compounds, but that the digestion method should be carefully selected with a consideration for the content of organometallic cation.

Abbreviations

ICP-AES: Inductively coupled plasma atomic emission spectrometer;
TMDW: Trace metals in drinking water

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Authors' contributions

J-SR and W-JS designed the study and led the writing of the manuscript. MJ conducted sample preparation and chemical analysis. All authors contributed equally to the data interpretation. All authors read and approved the final manuscript.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author (J.-S. Ryu, jongsikryu@pknu.ac.kr).

Competing interests

The authors declare that they have no competing interests.

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