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Research Article

CRITICAL EVALUATING FOR FIVE DIGESTION METHODS USING ICP-MS

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ABSTRACT
The metals Al, As, B, Br, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Ru, Sn, V and Zn and the non-metal Se are considered ''trace elements''(TE) because of their essentiality and very limited quantity in humans. Therefore, this study aims to understand the heavy metal contents in human biological materials, using different digestion methods and to recommend the most appropriate digestion method making this measurement. Three reference materials from different sources were selected to be digested by five methods to determine the contents of these trace elements by ICP-MS. The five digestion methods were nitric acid, nitric acid - hydrogen peroxide, nitric - sulfuric acid, nitric–perchloric acid and sulfuric acid methods. Analytical results indicated that the nitric acid procedure was the most efficient for recovering Br, Ca, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn from most certified reference materials. The sulfuric acid procedure yielded the lowest recovery of Pb from the certified reference material. The nitric acid procedure was recommended as the method for digesting the human biological materials samples herein, based on recovery analysis, cost and time taken. Nitric–perchloric acid procedure was not recommended because perchloric acid is potentially hazardous during digestion and it recovers relatively little heavy metal.
Keywords: heavy metal, reference materials, digestion method, Nitric acid

INTRODUCTION

Trace elements are essential components of biological structures, but at the same time they can be toxic at concentrations beyond those necessary for their specific biological functions. They most commonly function as essential coenzymes and cofactors for metabolic reactions and thus help and support basic cellular reactions required to maintain energy production and life. Even moderate deficiencies can lead to serious disease states.[1,2] Many analytical detection techniques, like AAS, AES, voltammetry, potentiometry, spectrophotometry and inductively coupled plasma mass spectrometry (ICP-MS) require a sample (analyte) to be present in a dissolved form. Using these detection techniques for trace elements, dissolution of matrix prior to the determination is a vital stage. Since the goal of the analysis is the determination of the total contents of elements present in the matrices in trace amounts, complete dissolution should be achieved. This means that a separation step (filtration, centrifugation) is not allowed and is not part of the digestion procedure. In the literature[3-8] much information can be found about the dissolution and decomposition of many combinations of matrices and analytes. Some standard reference materials, similar to a compost matrix, have been used to elucidate the recovery of heavy metals by different digestion methods[9,10]. (However, little attention has been paid to samples of popularly used composts. No standard official methods of digestion of composts exist in Egypt to measure amounts of heavy metals. Therefore, the aims of this study are to (1) evaluate the contents of Cd, Cr, Cu, Mn, Ni, Pb and Zn in seven human biological materials using different digestion methods; (2) explain the dissolution by the digestion methods, using inorganic acid mixtures, of human biological materials, and (3) recommend the most appropriate digestion method for determining the seven heavy metals in the various biological materials.

MATERIALS AND METHODS

Methods

The study samples

Three certified reference materials were used in this study. They were IAEA-A-13, Bowen's Kale, and GBWO7601. All these samples were prepared by recommended method from IAEA.

Methods of Digestion

Before digestion to analyze heavy metals, each sample was dried at 65^{o}c for 48 h; five digestion methods were applied herein, involving nitric acid[11], nitric acid - hydrogen peroxide[12], nitric - sulfuric acid[13], nitric–perchloric acid[14] and sulfuric acid[15]. All methods were performed in triplicate for each sample. From the reagents used for the oxidation, HNO_3 is the only acid which can be used alone. Use of this acid has several advantages:

- It is available in high purity,
- Nitrates are very soluble,
- It may be employed over a range of temperatures; it is active already at room temperature and disrupts organic materials, while at high temperature and pressure almost complete mineralization can be achieved.

Preparation of samples

The Certified Reference Materials of Human Hair (GBW07601), IAEA- A-13 (freeze dried animal blood from IAEA) and Bowen's Kale (dried leaves of Kale) from (International union of pure and applied chemistry) were used for accuracy evaluation.

Heavy metal analysis

The concentrations of Br, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Rb, S, Se and Zn in the final solutions were determined by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Quality assurance and quality control

Three standard reference materials, including IAEA- A-13 (freeze dried animal blood) from IAEA, Bowen's-Kale (Dried leaves of Kale) from (International union of pure and applied chemistry) Certified Reference Material of Human Hair (GBW07601) were digested in triplicate and analyzed using the five methods to support quality assurance and control (QA/QC). Table 1 a, b and c show the recoveries of heavy metals in the three standard reference materials by five digestion methods. The recoveries of eighteen elements by the five methods ranged from 62 % to 114 %, respectively. A blank was run for each digestion procedure to correct the measurements. For sets of every ten samples, a procedure b lank and spike sample, involving all reagents, was run to check for interference and cross contamination. Table 2 lists the method detection limits (MDL) of the five digestion procedures.

Statistical Analysis

Significant differences between concentrations of heavy metals, following different digestion methods were analyzed by ANOVA[16]. Statistical significance was defined as $p < 0:05$.

RESULTS AND DISCUSSION

Comparison of different digestion methods

Cadmium

The concentrations of Cd were higher in the RBC than in all other biological samples (Table 3). No significant ($p < 0:05$) differences existed between five digestion methods in terms of the recovery of Cd. Of the five digestion methods, nitric acid was the most efficient in recovering Cd from all biological samples. However, less Cd was recovered from most samples digested by sulfuric acid procedure was less than that recovered by other methods of digestion.

Chromium

The highest concentration of Cr was found by all digestion methods in the fingernails (Table 4). Nitric acid recovered significantly more Cr from all samples, than did any the digestion methods. Chromium exists in a number of oxidation states, and the most stable and common forms are Cr (VI) and Cr (III). These two forms have very different chemical properties, and Cr (VI) is more toxic and more readily extracted from solid particles, while Cr (III) is less mobile and adsorbed tightly onto siliceous materials[17]. The sulfuric acid Procedure still recovered the least Cr from most biological samples. The nitric acid and nitric–perchloric acid procedures did not significantly differ in terms of recovering Cr.

Copper

The Cu content was highest in serum, regardless of the digestion method used. However, nitric–perchloric acid and sulfuric acid procedures were less efficient than nitric acid procedures in recovering Cu from all biological samples (Table 5). During the nitric–perchloric acid procedure, adding $HClO_4$ accelerated the digestion. Copper has a high affinity for organic compounds[18], so using a digestion agent with a stronger oxidizing capacity results in the more complete decomposition the sample. Therefore, more Cu was recovered in digestion procedures in which stronger oxidants, such as $HClO_4$ and H_2SO_4 were involved. However, $HClO_4$, when hot, is a strong oxidant and can react with explosive force when brought into contact with easily oxidizable compounds, especially if the digestion mixture is almost dry.

Manganese

Manganese is relatively abundant in the samples used in this study (Table 6). The recovery of Mn in the samples is affected not only by the digestion method, but also by the type of sample.

Nickel

The five digestion methods did not significantly differ in terms of the recovery of Ni (Table 7). The nitric acid procedure showed more Ni in the samples, than any other procedure.

Lead

The sulfuric acid procedure showed poor recovery from the samples (Tables 8), probably because of the precipitation of Pb and the potential interference of $PbSO_4$ during the subsequent analysis. Relatively insoluble $CaSO_4$ may be formed, lowering Pb by co precipitation from the compost sample with higher Ca content. However, better dissolution is exhibited by the other methods, especially nitric acid digestion, which is associated with the lowest costs of reagents and equipment.

Zinc

Zinc was the most abundant heavy metal in blood components, according to all digestion methods. However, the methods clearly determined widely different Zn contents (Table 9). No digestion method recovered the most Zn from all samples, but nitric and nitric + hydrogen peroxide was recommended for recovering more Zn from most biological samples than the other methods.

Recommended Digestion Method

The nitric acid procedure was the most efficient method for recovering Br, Ca, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn from most biological samples herein. Nitric and perchloric acid was the most efficient in recovering Cu and was not the least efficient poorest method for recovering any of the other elements. As determined the certified reference material, the sulfuric acid procedure recovered the least Pb (Table 1). However, the sulfuric acid procedure recovered least Pb from most of the samples with potentially high levels of Ca. Consequently, the sulfuric acid procedure should not be used to determine the Pb content in compost with a high Ca content obtained by precipitation. The observed total Zn contents in all samples, determined by these five methods, differed greatly, implying the wide range of amounts of metals in the collected biological samples. HNO_3 was used to prevent explosive reactions of the organic matter with $HClO_4$ prior to the sample was digested. In summary, the nitric acid procedure is recommended as the method for digesting samples, based on recovery analysis and cost and time effectiveness.

CONCLUSION

Although all the samples showed that, nitric acid digestion was the most efficient in terms of the recovery of the heavy metals in this study, especially based on cost and time effectiveness, the nitric acid procedure was recommended as the standard method for digesting biological samples in a traditional open-vessel digestion system.

Table 1a: The recovery analysis of heavy metal content (µg/g)* in IAEA-A-13 (recovery %)

Element	Certified value	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Br	22.00	17.82 (81 %)	17.86 (81 %)	17.60 (80 %)	17.75 (81 %)	18.12 (82 %)
Ca	286.00	225.94 (79 %)	223.08 (78 %)	220.22 (77 %)	223.12 (78 %)	231.56 (81 %)
Cd	0.07	0.06[b] (92 %)	0.06 (91 %)	0.06 (88 %)	0.06 (90 %)	0.06 (86 %)
Cr	0.26	0.28 (108 %)	0.27 (105 %)	0.20 (76 %)	0.24 (92 %)	0.23 (89 %)
Cu	4.30	3.87 (90 %)	3.91 (91 %)	3.53 (82 %)	3.71 (86 %)	3.92 (91 %)
Fe	2400	1968.00 (82 %)	1920.00 (80 %)	1944 (81 %)	1961 (82 %)	1983 (83 %)
Hg	0.16	0.15[c] (91 %)	0.14 (90 %)	0.14 (90 %)	0.14 (91 %)	0.15 (91 %)
K	2500	2075 (83 %)	2074 (83 %)	2050 (82 %)	2061 (82 %)	2070 (83 %)
Mg	99.00	79.20 (80 %)	78.21 (79 %)	77.22 (78 %)	78.23 (79 %)	78.21 (79 %)
Mn	3.60	3.10 (86 %)	3.11 (86 %)	3.24 (90 %)	3.19 (89 %)	3.29 (91 %)
Na	12600	11844 (94 %)	11970 (95 %)	12096 (96 %)	12000 (95 %)	12062 (96 %)
Ni	1.00	0.78 (78 %)	0.77 (77 %)	0.73 (73 %)	0.75 (75 %)	0.72 (72 %)
P	940.00	761.40 (81 %)	761.40 (81 %)	752 (80 %)	755 (80 %)	770 (82 %)
Pb	0.18	0.13 (72 %)	0.13 (71 %)	0.11 (62 %)	0.12 (67 %)	0.14 (78 %)
Rb	2.30	1.63 (71 %)	1.61 (70 %)	1.61 (70 %)	1.62 (70 %)	1.65 (72 %)
S	6500	5070 (78 %)	4940 (76 %)	7410 (114 %)	6932 (107 %)	5213 (80 %)
Se	0.24	0.19 (80 %)	0.19 (79 %)	0.19 (78 %)	0.19 (79 %)	0.19 (81 %)
Zn	13.00	10.53 (81 %)	10.54 (81 %)	11.44 (88 %)	11.00 (85 %)	11.71 (90 %)

All recommended values are expressed on a dry weight basis at the time of analysis, the actual value is 0.064 and the actual value is 0.146

Table 1b: The recovery analysis of heavy metal content (µg/g)* in BOWEN'S KALE (recovery %)

Element	Certified value	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Br	24	19.44 (81 %)	19.23 (80 %)	18.78 (80 %)	19.11 (78 %)	19.52 (81 %)
Ca *	4.14	3.27 (80 %)	3.23 (78 %)	3.29 (79 %)	3.29 (79 %)	3.34 (81 %)
Cd	0.89	0.83 (93 %)	0.81 (91 %)	0.79 (89 %)	0.81 (91 %)	0.76 (85 %)
Cr	0.31	0.34 (110 %)	0.33 (106 %)	0.24 (78 %)	0.29 (94 %)	0.28 (89 %)
Cu	4.9	4.45 (91 %)	4.41 (90 %)	3.97 (81 %)	4.31 (88 %)	4.51 (91 %)
Fe	115	95.45 (83 %)	90.85 (79 %)	93.15 (81 %)	94.30 (82 %)	96.02 (83 %)
Hg	0.18	0.16 (91 %)	0.16 (90 %)	0.16 (90 %)	0.16 (91 %)	0.16 (91 %)
K *	2.43	2.02 (83 %)	2.00 (83 %)	2.13 (88 %)	2.07 (86 %)	2.13 (88 %)
Mg *	0.16[a]	0.13 (81 %)	0.12 (79 %)	0.14 (88 %)	0.14 (84 %)	0.13 (79 %)
Mn	15	13.10 (87 %)	13.11 (87 %)	13.65 (91 %)	13.38 (89 %)	13.49 (90 %)
Na*	0.23	0.22 (94 %)	0.22 (95 %)	0.22 (96 %)	0.22 (95 %)	0.22 (96 %)
Ni	1.08	0.86 (80 %)	0.87 (81 %)	0.79 (73 %)	0.83 (77 %)	0.80 (74 %)
P *	0.45	0.36 (81 %)	0.37 (82 %)	0.35 (78 %)	0.36 (79 %)	0.37 (83 %)
Rb	52	37.11 (71 %)	36.85 (71 %)	36.00 (69 %)	36.51 (70 %)	37.44 (72 %)
Se	0.14	0.11 (80 %)	0.11 (79 %)	0.12 (86 %)	0.11 (82 %)	0.11 (81 %)
Zn	31	25.73 (83 %)	26.04 (84 %)	27.59 (89 %)	26.71 (86 %)	28.21 (91 %)

% by weight, the actual value is 0.156

Table 1c: The recovery analysis of heavy metal content (µg/g) in (Certified Reference Material of Human Hair (GBW07601 (recovery %)

Element	Certified value	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Ca *	2.90	2.34 (81 %)	2.29 (79 %)	3.29 (79 %)	3.32 (80 %)	2.35 (81 %)
Cd	0.11	0.10 (93 %)	0.10 (91 %)	0.10 (89 %)	0.10 (91 %)	0.09 (85 %)
Cr	0.37	0.40 (108 %)	0.39 (106 %)	0.29 (78 %)	0.35 (94 %)	0.33 (89 %)
Cu	10.60	9.75 (92 %)	9.54 (90 %)	8.69 (82 %)	9.33 (88 %)	9.75 (91 %)
Fe	54	45.36 (84 %)	43.20 (80 %)	44.28 (82 %)	44.80 (83 %)	44.84 (83 %)
Mg	360	291.61 (81 %)	288.00 (80 %)	316.82 (88 %)	306.10 (85 %)	284.41 (79 %)
Ni	0.83	0.67 (80 %)	0.67 (80 %)	0.61 (73 %)	0.65 (77 %)	0.61 (74 %)
Pb	8.80	7.22 (82 %)	7.22 (82 %)	6.95 (79 %)	6.95 (79 %)	7.39 (84 %)
Zn	190	157.72 (83 %)	159.64 (84 %)	169.14 (89 %)	163.44 (86 %)	172.91 (91 %)

mg/g

Table 2: Method detection limits (µg /g) for different digestion methods of ICP-MS in this study

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Cd	0.006	0.007	0.004	0.006	0.004
Cr	0.08	0.08	0.10	0.08	0.07
Cu	0.02	0.04	0.04	0.04	0.03
Fe	0.008	0.006	0.006	0.008	0.007
Hg	0.006	0.008	0.004	0.005	0.004
K	0.01	0.008	0.006	0.01	0.01
Mg	0.06	0.05	0.04	0.06	0.04
Mn	0.05	0.04	0.06	0.05	0.06
Na	0.01	0.008	0.006	0.01	0.01
Ni	0.03	0.02	0.03	0.02	0.02
P	0.01	0.008	0.006	0.01	0.01
Pb	0.10	0.08	0.06	0.07	0.05
Se	0.008	0.006	0.006	0.008	0.007
Zn	0.02	0.03	0,07	0.04	0.03

Table 3: Cadmium contents (ng/g)* in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	1.81	1.72	1.51	1.66	1.53
Fingernails	0.22	0.21	0.18	0.20	0.19
Toenails	0.59	0.56	0.49	0.54	0.50
Whole blood[a]	3.12	2.96	2.62	2.86	2.64
Serum[a]	2.01	1.91	1.68	1.84	1.72
RBC[a]	8.39	7.96	7.12	7.69	7.21
Urine[a]	2.19	2.09	1.83	2.01	1.85

By weight, µg/L

Table 4: Chromium contents (ng/g)* in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	1.98	1.88	1.66	1.82	1.68
Fingernails	2.46	2.34	2.06	2.26	2.09
Toenails	2.44	2.32	2.04	2.25	2.06
Whole blood[a]	0.62	0.59	0.52	0.57	0.53
Serum[a]	0.33	0.31	0.28	0.30	0.28
RBC[a]	0.14	0.13	0.12	0.13	0.12
Urine[a]	0.41	0.39	0.34	0.38	0.35

By weight, µg/L

Table 5: Copper contents (ng/g)* in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	17.15	16.28	14.38	15.76	14.55
Fingernails	8.22	7.81	6.89	7.55	6.97
Toenails	4.72	4.48	3.96	4.34	4.09
Whole blood[a]	1180.23	1120.11	989.41	1084.36	1001.10
Serum[a]	1207.12	1145.77	1012.05	1109.17	1024.01
RBC[a]	1200.11	1139.12	1006.34	1102.72	1018.08
Urine[a]	44.27	42.02	37.12	40.68	37.59

By weight, µg/L

Table 6: Manganese contents (µg/g)* in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	2.65	2.53	2.21	2.44	2.25
Fingernails	3.11	2.97	2.62	2.86	2.64
Toenails	2.61	2.49	2.19	2.40	2.23
Whole blood[a]	8.13	7.65	6.80	7.50	6.91
Serum[a]	0.87	0.83	0.73	0.81	0.75
RBC[a]	0.54	0.53	0.45	0.51	0.46
Urine[a]	0.81	0.77	0.68	0.76	0.70

By weight, µg/L

Table 7: Nickel contents (ng/g)* in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	1.44	1.38	1.21	1.33	1.22
Fingernails	3.09	2.97	2.61	2.86	2.65
Toenails	2.73	2.63	2.31	2.52	2.32
Whole blood[a]	0.96	0.92	0.81	0.87	0.82
Serum[a]	0.95	0.91	0.80	0.81	0.80
RBC[a]	0.74	0.71	0.62	0.68	0.63
Urine[a]	2.26	2.18	1.92	2.11	1.93

By weight, µg/L

Table 8: Lead contents (ng/g)* in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	0.77	0.74	0.65	0.71	0.66
Fingernails	0.67	0.65	0.57	0.62	0.58
Toenails	0.66	0.64	0.56	0.61	0.57
Whole blood[a]	145.23	139.62	122.41	133.91	124.89
Serum[a]	43.10	41.42	36.38	40.11	37.21
RBC[a]	208.12	200.01	175.71	192.23	178.45
Urine[a]	10.29	9.91	8.70	9.51	8.82

By weight, µg/L

Table 9: Zinc contents (ng/g)[*] in 7 biological samples by five digestion methods

Element	Nitric acid	Nitric acid + H_2O_2	Sulfuric acid	Nitric and sulfuric acid	Nitric and perchloric acid
Hair	234.19	225.07	199.69	215.98	200.99
Fingernails	144.22	139.61	122.99	132.98	123.77
Toenails	124.55	119.41	105.97	114.88	123.77
Whole blood[a]	1000.01	961.87	852.87	922.35	858.52
Serum[a]	1145.11	1100.41	976,98	1056.64	982.77
RBC[a]	989.78	952.32	845.11	910.12	849.89
Urine[a]	411.23	395.46	351.28	379.44	353.09

By weight, μg/L

The sulfuric acid procedure typically recovered least of these heavy metals and should not be used to determine the Pb content in sample. Nitric–perchloric acid procedure is not recommended because perchloric acid is potentially hazardous during digestion and recovers relatively little heavy metal.

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