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Article

Fast Determination of Iron and Zinc in Hair and Human Serum Samples After Alkaline Solubilization by GF AAS

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Methods for the development and validation for determination of iron and zinc in human serum and hair samples by graphite furnace atomic absorption spectrometry (GF AAS) were performed. Solubilization was immediate by manual agitation in both samples with a 10 mL volume sample plus deionized water. Optimum pyrolysis and atomization temperatures were obtained by pyrolysis and atomization temperature curves in both matrices. For serum samples, the best temperatures were 1400 and 2500 °C (Fe) and 700 and 1600 °C (Zn), respectively. For hair samples, the best temperatures were 1000 and 2400 °C (Fe) and 800 and 1600 °C (Zn), respectively. Permanent modifiers and zirconium presented best the results for Zn in both matrices and for Fe in serum. Permanent modifier Nb was best for hair Zn. Serum and hair values were in agreement with the certified values for analytes and certified samples. The determined values for serum Fe and hair certified samples were 1.40 ± 0.2 and 114 ± 18 mg L^{-1}, respectively. For Zn certified reference materials (CRMs), the certified samples values were 1720 ± 32 μg L^{-1} and 172 ± 5 mg kg^{-1}. Simple, accurate and precise, this method represents a cost-effective detection protocol suitable for sample analysis for diagnosis of micronutrient malnutrition.

Keywords: Universol®, graphite furnace atomic absorption spectrometry, iron, zinc, hair, serum, permanent chemical modifiers

Introduction

The importance of optimal amounts of Fe for the survival of animals, plants and microorganisms is well established and human Fe deficiency is a worldwide problem resulting from a lack of proper dietary food intake.[1] A report from the World Health Organization estimates that 46% of the world's 5- to 14-year-old children and 48% of the pregnant women are anemic.[2] In addition, Fe has particularly relevant roles in neuronal and immune functions. Animal studies have revealed that feeding rats a low Fe diet early in life results in irreversible alterations of brain functions related to insufficient myelination and defective establishment of the dopaminergic tracts.[3]

Zinc is responsible for the operation of more than 200 enzymes, some of which are associated with the synthesis of DNA and RNA, and is also involved with immune system functions.[4] Zinc deficiency affects tens of millions of children throughout the developing world,[5] and has been implicated in the pathogenesis of stunting and impaired cognitive development[6] and infectious morbidity.[7]

The use of human serum in laboratory diagnosis of micronutrient deficiencies is well documented in the scientific literature.[8] Its composition is similar to plasma, but without fibrinogen and other substances that are used in the coagulation process. Human serum is recognized as a useful means for determining iron, copper, zinc and selenium levels in the body and for diagnosis and monitoring of specific diseases and the study of nutritional deficiencies.[9]

In addition to the diagnosis of specific micronutrient deficiencies, using blood serum as a biomarker, human hair has been employed by some authors as a "biological dosimeter",[10] "filament record"[11] or "a reflection of the environment"[12] to which an individual is exposed. This is so because if there is considerable exposure to a specific

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chemical element or drug via external contamination or through ingestion, over a period of time, the substance will be present in hair. The determination of trace elements in hair is not just a means of evaluating the current exposure, but also has the potential to evaluate and reconstruct past episodes relevant to health, even if the occurrence has ceased.[13]

Hair is a simpler matrix than blood and urine.[14] It is an attractive biological material due to its simplicity as a sample (easy to obtain, without trauma and/or discomfort), storage, transport and handling. Hair analysis is often used to verify possible poisoning or diagnose disease. Furthermore, the analysis of hair is easier because the analyte is present at a higher concentration than in blood and urine.[15-17]

There are a number of methods available in the scientific literature for Fe and Zn analysis in biological samples, specifically, blood serum and hair samples. Kandhro *et al*.[18] developed a cloud point extraction method applied for preconcentration of trace quantities of Zn and Fe in biological samples (serum and urine) of thyroid patients prior to determination by flame atomic absorption spectrometry (FAAS). The metals in serum and urine samples were complexed with 1-(2-thiazolylazo)-2-naphthol and entrapped in the surfactant octylphenoxypolyethoxyethanol (Triton X-114). After centrifugation, the surfactant-rich phase was diluted with 0.1 mol L^{-1} HNO_3 in methanol. Enrichment factors of 66.4 and 70.2 were obtained for the preconcentration of Zn^{II} and Fe^{III}, respectively. The obtained results showed sufficient agreement (> 98%) for Zn^{II} and Fe^{III} in certified reference materials (CRMs).

To evaluate trace metals such as Fe and Zn in hair, the dissolution of hair samples is necessary in order to determine these elements. A number of dry and wet ashing procedures are available. Friel and Ngyuen[19] evaluated these techniques and recommended dry ashing of hair samples to be analyzed for Cu, Mn and Zn, and wet ashing with HNO_3 for Fe assays. Several procedures for dissolving hair samples include HCl, HNO_3 or binary mixtures of HCl and HNO_3 with $HClO_4$. In another study, the authors standardized a dissolution procedure using HNO_3 and H_2O_2 for the determination of Fe, Co, Ni, Mn, Zn, Cu, Cd, and Pb using inductively coupled plasma atomic emission spectrometry (ICP-AES) in order to overcome the shortcomings which include corrosive effects.[19]

Although the above studies are valid and well recognized throughout the scientific literature, unfortunately, major drawbacks to the analysis of trace elements using such methods include the elevated costs of reagents and relatively elaborate time-consuming process in sample preparation. Therefore, there is an urgent need for a method that is fast, accurate, safe, and cost effective.

The use of a new solubilizing alkaline agent of organic and non-organic material, called Universol®,[20] developed by our scientific group, has been shown to be a safe and simple reproducible method procedure, promoting the total solubilization, with (vortex) or without agitation, very quickly (usually within 1-5 min), at room temperature and without the need of any acid digestion nor microwave or ultrasound irradiation. The dissolution is immediate and forms a homogeneous and indefinitely stable solution. Our experience with serum, urine and whole blood analysis for some analytes was performed diluting serum samples with 1.0% v/v nitric acid plus 0.1% v/v of cetyltrimethylammonium chloride (CTAC) or Triton X-100.[21-28]

Since the two elements investigated in this study are internal transition elements, we investigated the carbide forming permanent modifier (500 µg) group members (carbide forming elements) niobium, tantalum, titanium and zirconium. Moreover, the two matrices (whole blood and serum samples) have been studied in previous works.[29,30] They are rich in carbon and tend to form carbonaceous residues within the graphite tube (to the point that they interfere with light passage from the lamps, thus interfering with obtained absorbance). The use of carbide forming elements aids in preventing the formation of these residues within the graphite tube.

This study investigates the methods for iron and zinc determination in serum and hair samples using graphite furnace atomic absorption spectrometry (GF AAS) after dissolution with Universol®, which presented the advantage of immediately dissolving and forming a homogeneous and indefinitely stable solution.

Experimental

Instruments and apparatus

A Perkin Elmer Aanalyst™ 400 atomic absorption spectrometer (Norwalk, USA), equipped with graphite furnace (HGA800) with background correction by deuterium lamp and autosampler (AS-800) was used for iron and zinc quantification. Readings were made for integrated absorbance (peak area). A multi-element hollow cathode lamp (HCL) for Fe, Zn, Mn and Cu (Perkin Elmer Part No. N305-0212) was used according to the recommended manufacturer's instructions. The wavelengths (nm), slits (nm) and applied current (mA) were of 248.3, 1.8/1.35 and 15 for Fe and 213.9, 2.7/1.8 and 15 for Zn, respectively. The graphite furnace autosampler received an injected volume of 20 µL for samples of both analytes and solutions used for calibration.

High purity argon was used as inert gas (99.996%, White Martins, Belo Horizonte, MG, Brazil). Pyrolytic graphite tubes with an L'Vov platform (Perkin Elmer, Part No. B3001264 and B3001263) were used for all the studies. Adjustable micropipettes of 20-200 and 100-1000 µL (Digipet, Curitiba, PR, Brazil) were used to prepare the solutions.

Reference solutions and reagents

The reagents used in this study were of analytical purity. All aqueous solutions were prepared using deionized water (resistivity of 18.2 MΩ cm) obtained by a direct-Q system (Millipore, Billerica, MA, USA). Universol®, benzyltrimethylammonium hydroxide 40% m/v in water (patent number PI 1003893-0)[20] was acquired from Sigma-Aldrich (St. Louis, MO, USA, No. 100974415). Nitric acid (Merck, Darmstadt, Germany, No. 7587956) was used to prepare the standard Fe and Zn solutions. Zirconium (Aldrich, No. 27497-6), niobium (Fluka, St. Louis, USA, No. 274917), titanium (Aldrich, 03,796 in-1EA) and tantalum (Fluka, No. 86275) solutions were prepared at 1 mol L^{-1} in hydrochloric acid and were used to obtain the permanent modifiers. A concentration of 1000 mg L^{-1} of tungsten was also used and prepared by dissolving 0.2 g of Na_2WO_4 (Merck, No. 106672) in 100 mL of deionized water. The iron and zinc analytical solutions were prepared from stock solutions of 1000 mg L^{-1} prepared using 5% v/v nitric acid from ampoules (Titrisol, Merck) and stored according to the manufacturer's instructions.

Washing hair samples

Currently, the washing method, developed by the International Atomic Energy Agency (IAEA), is commonly used in many laboratories.[29-32] Hair samples were treated over several stages: in 20 mL of acetone, twice in 40 mL of twice distilled water, and finally, once again in 20 mL of acetone. After washing, the samples were dried at 80 °C and weighed (about 100 mg).

Platform treatment with permanent modifiers

Each L'Vov platform inserted in a graphite tube was treated with the permanent modifiers (tungsten, zirconium, titanium, tantalum and niobium) using a specific heating program (Table 1), according to da Silva *et al.*[33] Volumes of 50 µL of each modifier were injected by the autosampler pipette in the L'Vov platform. This tube was then submitted to a heating temperature program and the procedure was repeated 10 times to result in a platform treated with 500 µg of permanent modifier.

Table 1. Temperature program for treatment of graphite tubes with permanent modifiers

Step	Temperature / °C	Ramp / s	Hold / s	Ar flow rate / (mL min^{-1})
1	90	5	15	250
2	140	5	15	250
3	1000	10	10	250
4	2000	0	5	0
5	20	1	10	250

Samples and sample preparations

The analyzed serum and hair samples were collected from 20 pre-adolescent, menstruating girls enrolled in a public elementary school in Belo Horizonte, located in the Brazilian state of Minas Gerais as part of a larger, controlled and randomized clinical trial.[34] One sample was used in the methods optimization study. Preparation of the sample was quite simple: 500 µL of serum was added to 500 µL of Universol®, while about 100 mg of dried hair samples were added to 500 µL of Universol®. Solubilization is immediate and the expected high values encountered from these metals in serum and human hair from the resulting solutions were diluted to 10 mL with ultrapure water.

For accuracy, CRM was tested against the serum samples using Trace Elements Serum L^{-1} from Seronorm (Sero, Billingstad, Norway) and human hair reference material (IAEA-086, International Atomic Energy Agency, Vienna, Austria) was tested against the hair samples.

LOD and LOQ calculation

The limit of detection (LOD) and limit of quantification (LOQ) for both analytes (Fe and Zn) were calculated after 10 measurements of the Universol® plus water in a final volume of 10 mL. The LOD was calculated as 3s / a, while the LOQ was 10s / a, where s is a standard deviation of ten measurements of the blank and a is the slope of the aqueous calibration curve.

Results and Discussion

Analytical parameters of merit

Comparison of the average slopes of three aqueous calibration curves and the average slopes of three matrix matching calibration curves for serum and hair samples,

and for both analytes, resulted in no significant difference, which was in agreement with results from the F-test and Student's *t*-test analysis.

To choose the best modifier element, that is to say, a modifier element that promotes increased sensitivity with a symmetrical peak, a low relative standard deviation (RSD) and corrected background, using the manufacturer's recommended furnace program, studies were performed with a sample prepared, as shown above, in each tube treated as described. For both metals, the best modifier (taking into account the results obtained for sensitivity, pulse shape of absorption, background correction, and RSD for three replicate studies less than 5%) was permanent Zr (500 µg) deposited on a L'Vov platform inserted into a graphite tube.

To obtain optimum temperatures for pyrolysis and atomization of each analyte in the studied matrices and prepared by solubilization with Universol®, temperature curves were performed for pyrolysis and atomization in each matrix (serum and hair diluted as described previously), using Zr as a permanent modifier for Fe and Zn in serum and Zn in hair. To analyze Fe in hair, the permanent modifier Nb (500 mg) was used. Results from analyzing diluted serum samples demonstrated that optimum temperatures curves obtained for the pyrolysis and atomization (better sensitivity, symmetrical peak and background correction) were 1400 and 2500 °C for iron and 700 and 1600 °C for zinc, respectively. According to results for the hair samples, the best temperatures were 1000 and 2400 °C for iron (using permanent Nb, 500 µg) and 800 and 1600 °C for zinc (using permanent Zr, 500 µg), respectively. For all cases, the values for background were below 0.1 absorbance integrated units (s). The temperature program used for the determination of iron and zinc in serum and hair is presented in Table 2. Figures 1 and 2 present the absorption signals for Fe in serum and hair, respectively. As can be observed in the Figures, the shape of the absorption signals was very different for serum and hair. This may be explained by the differences of the best pyrolysis temperatures obtained for iron in serum and hair samples (Figures 1 and 2).

Analytical parameters of merit

Comparison of the average slopes of three aqueous calibration curves and the average slopes of three matrix matching calibration curves for serum and hair samples, and for both analytes, resulted in no significant difference, which was in agreement with results from the F-test and Student *t*-test analysis. Thus, the determination of both analytes from both matrices can be performed against aqueous curves. The parameters of merit of the aqueous

Table 2. Temperature program for the determination of Fe and Zn in serum and hair samples by GF AAS

Step	Temperature / ºC	Ramp / s	Hold / s	Ar flow rate / (mL min^{-1})
Dry	100	5	20	250
Dry	140	15	15	250
Pyrolysis	1400,[a] 700,[b] 1000,[c] 800[d]	10	20	250
Atomization	2500,[a] 1600,[b] 2400,[c] 1600[d]	0	5	0 (read)
Clean	2600	1	5	250

[a]Fe in serum; [b]Zn in serum; [c]Fe in hair; [d]Zn in hair.

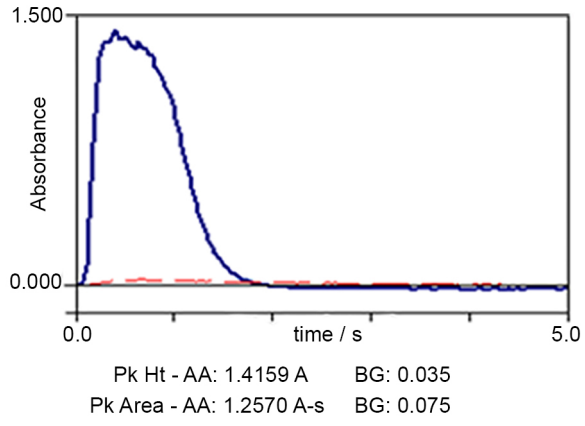

Figure 1. Absorption signal for 1.7 pg of Fe in serum sample using Zr (500 µg) as the permanent modifier.

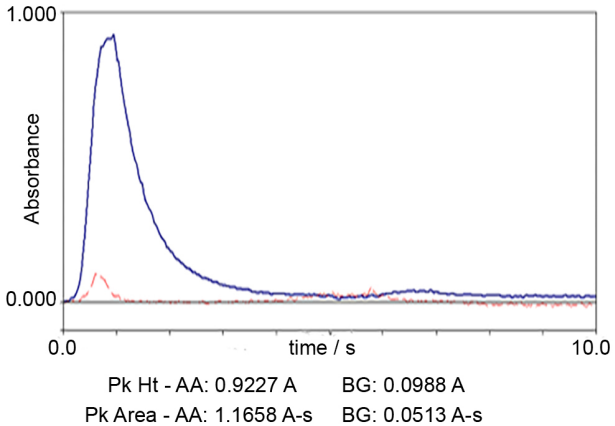

Figure 2. Absorption signal for 1.6 ng of Fe in hair sample using Nb (500 µg) as permanent modifier.

calibration curves for both analytes presented an $r^2 > 0.996$ result. Other figures of merit, determined by this method, are presented in Table 3.

Table 3. Analytical parameters of merit

Parameter	Iron	Zinc
r^2	0.9968	0.9992
Characteristic mass[a] / pg	5.8 ± 0.6	9.6 ± 0.8
LOD (serum) / (μg L^{-1})	0.7	0.5
LOD (hair) / (μg kg^{-1})	0.3	0.1
LOQ (serum) / (μg L^{-1})	2.3	1.7
LOQ (hair) / (μg kg^{-1})	1.0	0.3
Precision[b] (RSD, n = 3) / %	< 1.2	< 0.8
Accuracy of Trace Elements Serum L-1[c] / (mg L^{-1})	1.40 ± 0.2	1.720 ± 0.032
Accuracy of trace elements in human hair IAEA-086[d] / (mg kg^{-1})	114 ± 18	72 ± 5

[a]Recommended values: Fe = 5.0 pg and Zn = 10.0 pg; [b]concentration of 1.9 mg L^{-1} of Fe and 7.7 mg L^{-1} of Zn; [c]certified serum values: 1.4 ± 0.1 mg L^{-1} for Fe and 1742 ± 82 μg L^{-1} for Zn; [d]certified hair values: 123 ± 13 mg kg^{-1} for Fe and 167 ± 8 mg kg^{-1} for Zn. r^2: coefficient of determination; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

The characteristic masses determined for iron and zinc were close to the values recommended by the equipment manufacturer: for Fe, observed characteristic mass was 5.8 ± 0.6 pg (recommended is 5 pg) and for Zn, 9.6 ± 0.8 pg (recommended is 10 pg).

The LODs were 0.7 and 0.5 μg L^{-1} for Fe and Zn in serum samples, respectively. For hair, the LODs were 0.3 and 0.1 ng g^{-1} for Fe and Zn, respectively. The obtained LOQs were 2.3 and 1.7 μg L^{-1} in serum and 1.0 and 0.3 ng g^{-1} in the hair sample for Fe and Zn, respectively. The obtained LOD and LOQ values were also suitable for determination by GF AAS and compatible with other methods according to the literature. The blanks of Universol® plus water (in a final volume of 10 mL) were lower than 0.008 for both analytes.

In comparison with the serum certified reference material, the obtained values were 1.40 ± 0.2 mg L^{-1} for Fe (Fe certified value of 1.43 ± 0.1 mg L^{-1}) and 1720 ± 32 μg L^{-1} for Zn (Zn certified value of 1742 ± 82 μg L^{-1}), while analyzing the hair sample certified reference material, the observed results were 114 ± 18 mg kg^{-1} for Fe (certified value of 123 ± 13 mg kg^{-1}) and 172 ± 5 mg kg^{-1} for Zn (certified value of 167 ± 8 mg kg^{-1}). The F- and *t*-tests (Student's, 95% confidence level) showed no difference between these values. Therefore, this demonstrates good accuracy for the proposed methods.

Analytical application

Tables 4 and 5 present the results employing the proposed study methodologies for the determination of Fe and Zn in serum and hair of 20 pre-adolescent, menstruating girls. The obtained results were between 0.6 to 2.1 μg L^{-1} for Fe and 2.8 to 11.3 μg L^{-1} for Zn in serum samples and 13.9 to 25.8 μg g^{-1} for Fe and 120.6 to 191.2 μg g^{-1} for Zn in hair samples.

Table 4. Iron and zinc levels (n = 3), in serum from pre-adolescent, menstruating girls in schools in the state of Minas Gerais, Brazil

Sample	Iron		Zinc	
	Concentration / (mg L^{-1})	RSD (n = 3)	Concentration / (mg L^{-1})	RSD (n = 3)
1	1.32	0.05	6.91	0.30
2	1.36	0.09	6.90	0.12
3	1.24	0.02	2.82	0.15
4	1.31	0.03	2.75	0.22
5	0.66	0.06	7.27	0.33
6	0.70	0.04	7.17	0.18
7	0.96	0.10	7.84	0.40
8	1.08	0.08	7.68	0.80
9	0.80	0.06	6.29	0.06
10	1.96	0.12	6.61	0.08
11	1.88	1.11	6.65	0.09
12	1.53	0.09	6.96	0.21
13	1.57	0.41	6.99	0.18
14	1.06	0.13	7.95	0.38
15	1.12	0.51	8.08	0.23
16	1.09	0.92	6.80	0.06
17	0.98	0.33	6.90	0.15
18	1.16	0.16	6.50	0.11
19	1.22	0.18	6.52	0.16
20	1.53	0.66	6.83	0.12

RSD: relative standard deviation.

Kazi *et al.*[35] compared the level of essential trace elements chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) in biological samples (whole blood, urine, and scalp hair) of male and female patients with diabetes mellitus type 2 (n = 257) comparing them with their non-diabetic male and female controls (n = 166) aged 45 to 75 years. The concentrations of Zn, obtained by these authors in scalp-hair samples from the control subjects, ranged from 203.6-220.5 and 177.2-197.5 μg g^{-1}. However, in male patients, this range was 131.9 to 163.5 and 140.7 to 169.3 μg g^{-1} in age groups 45 to 60 and 61 to 75 years, respectively. Our work was done

Table 5. Iron and zinc levels (n = 3), in hair samples from pre-adolescent, menstruating girls in schools in the state of Minas Gerais, Brazil

Sample	Iron		Zinc	
	Concentration / (μg g^{-1})	RSD (n = 3)	Concentration / (μg g^{-1})	RSD (n = 3)
1	25.8	1.67	179.4	2.13
2	25.0	2.66	188.8	1.10
3	19.4	0.84	160.7	1.60
4	15.8	1.56	175.9	0.50
5	21.9	1.53	186.7	0.16
6	22.3	2.11	180.3	1.12
7	16.2	0.64	173.2	2.10
8	24.2	1.23	175.9	2.16
9	22.7	1.74	181.0	0.94
10	19.5	1.47	191.2	1.77
11	18.1	0.75	179.5	1.29
12	22.2	1.74	164.7	3.08
13	19.9	1.59	152.1	0.58
14	13.9	2. 07	130.2	1.20
15	18.2	1.80	120.6	0.98
16	19.9	1.58	147.7	0.57
17	23.1	2.50	169.7	2.58
18	15.2	2.06	175.6	0.37
19	18.2	0.86	176.0	1.26
20	15.0	0.56	188.0	3.76

RSD: relative standard deviation.

with young, menstruating girl subjects, and the obtained values for Zn in hair were approximately of 170 μg g^{-1}, a result that is in accordance with data cited by the authors.

A study by Baranowska *et al.*,[36] which analyzed hair and teeth samples collected from the inhabitants of Katowice, Gliwice, Pyskowice and Tychy (Silesia, Poland) for multi-element content by X-ray fluorescence spectrometry, obtained an average concentration of 36.3 μg g^{-1} for Fe and 162.1 μg g^{-1} for Zn in hair samples. In our study, the highest value observed was for iron in hair (25.8 μg g^{-1}). However, the author's data from the aforementioned study was taken from a contaminated area. Our results for Zn were very close to those obtained by the authors.

Izquierdo Alvarez *et al.*,[37] reviewing normal levels of Cu, Zn and Se in serum of pregnant women, determined an average Zn concentration of 6.5 ± 1.3 mg L^{-1}, while in our study, the observed values were very close to those of the authors': around 2.8 to 8.1 mg L^{-1}, with many values close to 6 mg L^{-1}, which was in agreement with the values found by these authors. Martín-Lagos *et al.*[38] determined Zn and Cu concentrations in serum from Spanish women during pregnancy. In pregnant women, Zn serum levels were 3-13.4 μg L^{-1}, whereas in a non-pregnant women group, the mean serum level was 9.5 ± 2.7 μg L^{-1}. These values are considered very low compared with our results and the others cited here.

Zaichick and Zaichick[39] studied the age dynamics and gender on major and minor trace element contents in scalp hair of 80 relatively healthy 15 to 55-year-old women and men by instrumental neutron activation analysis (INAA). The average iron and zinc concentrations were 88.2 ± 6.7 and 154 ± 3 μg g^{-1}, respectively. Our observed values were much lower than those cited by these authors for Fe (but in accordance with other studies), while for Zn, the results were very close. No correlation between age groups was observed by the authors.

Hatipoglu *et al.*[40] investigated the relationship between serum ghrelin levels and hair Zn concentrations in children. The children were divided into two groups according to hair Zn concentrations. Group 1 consisted of children with low (< 70 μg g^{-1}) hair Zn levels, and group 2 included children with normal (≥ 70 μg g^{-1}) hair Zn levels. The results obtained in our study were greater than 121 μg g^{-1} for Zn in hair samples. Hair Zn concentrations and serum ghrelin levels were measured in all children. There were 10 children with low hair Zn levels (group 1) and 15 with normal levels (group 2). Zn serum levels and ghrelin concentrations in group 1, 103.1 ± 71.8 and 1 ± 0.2 μg L^{-1}, respectively, were lower than in group 2, 164.9 ± 40.5 and 1.2 ± 0.2 μg L^{-1}, respectively.

Sreenivasa Rao *et al.*[41] determined Fe, Mn, Zn and Cu concentrations in hair samples of Indian residents and observed that iron levels (25-50 μg g^{-1}) increased with age. The levels obtained in the present work matches well with the values reported for Indian residents by other researchers. Similarly, the Zn content (ca. 100 μg g^{-1}) obtained from the Indian population group in these investigations matches well with another study conducted by the same authors. In our study, the values for Fe in hair were between ca. 14 to 26 μg g^{-1}, and for hair Zn, an average of ca. 170 ± 19 μg g^{-1} was observed. The LOD, calculated on the basis of 3 times the standard deviation of the blank, were 2 μg g^{-1} for Fe and 2.5 μg g^{-1} for Zn, while in our study, we obtained 0.3 and 0.1 μg kg^{-1}, respectively for Fe and Zn.

Finally, Ching-Chiang *et al.*[42] determined the Se, Fe, Cu and Zn levels in serum of patients at different stages of viral hepatic diseases. Obtained iron levels were 1.4 ± 0.4, 1.2 ± 0.3, 1.5 ± 0.4, 1.2 ± 0.4 and 1.0 ± 0.5 mg L^{-1} in healthy controls, Hepatitis B virus carriers, chronic hepatitis B, hepatic cirrhosis and hepatocellular carcinoma, respectively. While these authors had obtained 1.4 mg L^{-1} for the healthy controls, we observed an average Fe serum

of ca. 1.5 mg L^{-1} in young girls, a value that was relatively close to those obtained by these authors.

Conclusions

The developed methods done in this study are considered fast, efficient and inexpensive while facilitating the determination of Fe and Zn in serum in subjects at-risk for micronutrient malnutrition. The serum was solubilized using Universol® at a 1:1 proportion, and adequately diluting the sample according to the expected metal concentration. To determine iron and zinc, the serum solutions plus Universol® were diluted 10 times representing a dilution factor for serum 20 times for both metals. In hair samples, the dilution factor was also 20-fold for both metals. Of the modifiers investigated, permanent Zr (500 mg) was more efficient for both analytes in both matrices, except for Fe in hair, in which the best modifier proved to be permanent niobium (500 µg). Pyrolysis and atomization temperatures for the two analytes were optimized through temperature curves for pyrolysis and atomization in the matrix (serum or hair plus Universol® and water). The addition of Universol® to both serum and dry hair resulted in an instantaneous solubilization forming a homogeneous suspension and an indefinitely stable matrix reducing background absorption, a process not observed in preciously published studies. The accuracy (verified using certified reference materials) was in agreement with the certified values for both metals in both matrices. It is also important to note that the analysis of hair resulted in relative standard deviations, most of which were less than 3% with many values near or below 1%, demonstrating that hair and metals dissolve homogeneously in Universol®.

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References

1. Zimmermann, M. B.; Hurrell, R. F.; *Lancet* **2007**, *370*, 511.
2. http://apps.who.int/iris/bitstream/10665/66914/1/WHO_NHD_01.3.pdf?ua=1 accessed in September 2015.
3. Rouault, T. A.; *Nat. Rev. Neurosci*. **2013**, *14*, 551.
4. Livingstone, C.; *Nutr. Clin. Pract*. **2015**, *30*, 371.
5. Fischer, W. C. L.; Ezzati, M.; Black, R. E.; *J. Clin. Nutr*. **2009**, *63*, 591.
6. Grantham-McGregor, S.; Cheung, Y. B.; Cueto, S.; Glewwe, P. L.; Richter, L.; Strupp, B.; *Lancet* **2007**, *369*, 60.
7. http://www.cdc.gov/nutritionreport/pdf/Nutrition_Book_complete508_final.pdf#zoom5100 accessed in September 2015.
8. Pedraza, D. F.; Rocha, A. C.; Sales, M. C.; *Ciênc. Saúde Colet.* **2013**, *18*, 3333.
9. de Oliveira, A. P.; *Rev. Anal*. **2006**, *25*, 76.
10. Tepe Çam, S.; Polat, M.; Seyhan, N.; *Appl. Radiat. Isot*. **2014**, *94*, 272.
11. Abdulrahman, F. I.; Akan, J. C.; Chellube, Z. M.; Waziri, M.; *World Environ*. **2012**, *2*, 81.
12. Marques, R. C.; Bernardi, J. V.; Abreu, L.; Dórea, J. G.; *Arch. Environ. Contam. Toxicol.* **2015**, *68*, 432.
13. Arnold, W.; Sachs, H.; Fresenius, J.; *Anal. Chem.* **1994**, *348*, 484.
14. Preedy, V. R.; *Handbook of Hair in Health and Disease*, 1st ed.; Wageningen Academic Publishers: Wageningen, 2012.
15. Chatt, A.; Katz, S. A.; *Hair Analysis, Applications in the Biomedical and Environmental Sciences*, 1st ed.; VCH Publishers: New York, 1988.
16. Bencze, K.; Fresenius, J.; *Anal. Chem*. **1990**, *337*, 867.
17. Caroli, S.; Senofonte, O.; Violante, N.; Fornarelli, L.; Powar, A. J.; *Microchem. J.* **1992**, *46*, 174.
18. Kandhro, G. A.; Kazi, T. G.; Baig, J. A.; Afridi, H. I.; Shah, A. Q.; Sheikh, H. R.; Kolachi, N. F.; Wadhwa, S. K.; *J. AOAC Int*. **2010**, *93*, 1589.
19. Friel, J. K.; Ngyuen, C. D.; *Clin. Chem.* **1986**, *32*, 739.
20. da Silva, J. B. B.; Donnici, C. L.; *Br PI 1003893-0/WO 2,012,045,138*, **2012**.
21. Fabrino, H. J. F.; Silveira, J. N.; Borges, W.; Beinner, M.; Silva, J. B. B.; *J. Anal. Toxicol.* **2011**, *8*, 571.
22. Fabrino, H. J. F.; Borges, S. S. O.; Goes, A.; Silva, J. B. B.; *At. Spectrosc*. **2007**, *25*, 227.
23. Andrada, D.; Pinto, F. G.; Magalhães, C. G.; Nunes, B. R.; Franco, M. B.; Silva, J. B. B.; *J. Braz. Chem. Soc*. **2006**, *17*, 328.
24. Pinto, F. G.; Rey, U. V.; Ansaloni, L. M. S.; Andrade, D.; Silva, J. B. B.; *Ecletica Quim.* **2006**, *30*, 59.
25. da Silva, J. B. B.; Bejarano, R. O.; Demicheli, C.; Frezard, F.; *At. Spectrosc*. **2006**, *27*, 93.
26. Pinto, F. G.; Andrade, D.; Rey, U. V.; Ansaloni, L. M. S.; Silva, J. B. B.; *Anal. Lett.* **2006**, *39*, 2441.
27. Magalhães, C. G.; Lelis, K. L. A.; Rocha, A. C. R.; Silva, J. B. B.; *Anal. Chim. Acta* **2002**, *464*, 323.
28. Nunes, B. R.; Magalhães, C. G.; Silva, J. B. B.; *J. Anal. At. Spectrom*. **2002**, *17*, 1335.
29. Gurdus, A.; *J. Radioanal. Chem*. **1973**, *15*, 229.
30. Borella, P.; Rovesti, S.; Caselgrandi, E.; Bargellini, A.; *Mikrochim. Acta* **1996**, *123*, 271.
31. Cargnello, J. A.; Powell, J. J.; Thompsom, P. H.; Crocker, P. R.; *Analyst* **1995**, *120*, 783.

32. Bagliano, G.; *Anal. Chim. Acta* **1981**, *123*, 45.
33. da Silva, J. B. B.; Giacomelli, M. B. O.; Curtius, A. J.; *Microchem. J.* **1998**, *60*, 249.
34. Beinner *et al*., personal communications.
35. Kazi, T. G.; Afridi, H. I.; Kazi, N.; Jamili, M. K.; Arain, M. B.; Jalbani, N.; Kandhro, G. A.; *Biol. Trace Elem. Res*. **2008**, *122*, 1.
36. Baranowska, I.; Barchanski, L.; Bak, M.; Smolec, B.; Mzyk, Z.; *Pol. J. Environ. Stud.* **2004**, *13*, 369.
37. Izquierdo Alvarez, S.; Castañón, S. G.; Ruata, M. L.; Aragüés, E. F.; Terraz, P. B.; Irazabal, Y. G.; González, E. G.; Rodríguez, B. G.; *J. Trace Elem. Med. Biol.* **2007**, *21*, 49.
38. Martín-Lagos, F. M.; Navarro-Alaracón, C.; Terrés-Martos, H.; López-García de La Serrana, V.; Pérez-Valero, M. C.; López-Martínez, M.; *Biol. Trace Elem. Res*. **1998**, *61*, 62.
39. Zaichick, S.; Zaichick, V.; *Biol. Trace Elem. Res*. **2010**, *134*, 41.
40. Hatipoglu, N.; Atabek, M. E.; Kurtoglu, S.; Köklü, E.; *J. Clin. Res. Pediatr. Endocrinol.* **2008**, *1*, 1.
41. Sreenivasa Rao, K.; Balaji, T.; Prasada, R.; Babu, T. Y.; Naidu, G. R. K.; *Spectrochim. Acta, Part B* **2002**, *57*, 1333.
42. Ching-Chiang, L.; Jee-Fu, H.; Li-Yu, T.; Yeou-Lih, H.; *Biol. Trace Elem. Res*. **2006**, *109*, 15.

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