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Direct Determination of Aluminum and Zinc in Serum by Graphite Furnace Zeeman Effect Background Correction

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Abstract

This study was undertaken as a part of method modification and validation for rapid and accurate analysis of aluminum and zinc in serum samples. This paper reported an improved, precise and accurate graphite furnace atomic absorption spectrometric method using Zeeman Effect background correction for the direct determination of aluminum and zinc in serum. We considered 11 healthy volunteers children aged group 0-18 months and 64 children from poor-socioeconomic status of the same aged group.

Direct determination method includes small volume of sample (10 -50 µL), easy and quick sample preparation (30 min) and an optimized furnace program in order to obtain a justified characteristic mass (m_o). The analysis of zinc required magnesium nitrate [Mg $(NO_3)_2$] as a matrix modifier whereas aluminum was determined without any modifier. Optimal measuring condition was established for two modifiers and the most appropriate modifier was then chosen on the basis of the sensitivity and detection limits. The mean normal serum aluminum and zinc concentrations from 11 healthy subjects was 36.0 ± 9.21 µg/L and 1.80 ± 0.53 µg/L. The mean normal serum aluminum and zinc for the 64 was 78.60 ± 54.77 µg/L and 1.23 ± 0.88 µg/L.

The method has proved to be adequate for routine monitoring purposes due to its simplicity and high sensitivity. This study supported our project for routine screening of zinc and aluminum status in poor socio-economic populations especially those are victim of diarrheal disease. Our method validation project is continuing for various clinical samples like, urine and cerebrospinal fluid.

Keywords: Graphite Furnace, Zeeman-effect, Matrix Modifier, Serum, Aluminum and Zinc.

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1. Introduction

Determination of aluminum and zinc in serum samples by graphite furnace atomic absorption technique (GFAA) is performed routinely in hospitals, clinics, and in research laboratories. Accurate determination of the elements in biological materials has become of great importance, since these elements are suspected to be implicated in the etiology of various disorders [1]. The possible association of aluminum not only with metabolic bone disease, but also with encephalopathy, dictates caution when dealing with the pediatric population on long-term parenteral nutrition [2-3]. In the absence of reliable method for serum concentration information, it seems proper to monitor the serum aluminum and zinc concentration [4]. Since aluminum toxicity has been reported to impair the formation and release of parathyroid hormone [5].

Published reference values for aluminum in human body fluids vary widely [4], indicating the difficulties in this type of determination. Contamination reportedly is a serious problem. The direct method that includes centrifugation of the samples and measurement of aluminum in the supernate reportedly gives incorrect results, because the precipitate contained appreciable amounts of aluminum [6]. However such methods can be adequate for routine use in determining aluminum concentration in the biological fluids. Those direct methods that do not include a separation step are considered troublesome for several reasons: sample splutter during heating, formation of carbonaceous residues in the atomizers, difficulties in delivering reproducible amounts of sample, and volatile losses of aluminum as its chloride. Some of these difficulties can be overcome by diluting samples with deionized water, or 0.1% Triton X-100 [7].

Zinc has been recognized as an essential element, which takes part in metabolism. Changes in levels of serum zinc were discovered in individuals with hepatitis, anemia, hypertension, hyper-and hypothyroidism, nutritional disorders, allergy, etc.[8-10]. Zinc is considered to have an important immuno-regulatory effect on lymphocytes, lymph tissue, neutrophiles, macrophages, mastocytes, and platelets. Zinc deficiency is among the ten most important factors that lead to increased morbidity and mortality in developing countries [11-12]. Clinical manifestations of zinc deficiency in early childhood can lead to acute or chronic diarrhea with malnutrition, mental disorders, and behavioral problems. With age, in zinc deficiency one can observe alopecia, growth retardation, skin lesions, and common infections in children [13-14].

One of the major problems encountered in zinc determination is the control of contamination in all steps from sample collection and storage to the handling procedures preceding injection of the sample into the measuring device. A major source of zinc

contamination is human sweat [5] The accuracy, as estimated by analyzing serum with known amounts of zinc and aluminum, in this study was excellent, and the precision was *approx.* 2-5% RSD for day to day determination. Peak-area measurements were used in this procedure; as a consequence the quantitation was carried out by use of standards in 0.1% Triton X-100 calibration graph. The sample throughput is 20 h^{-1}, making the procedure well suited for routine clinical analysis [15]. With its good analytical parameters and accuracy, the applied Graphite Furnace method for measurement of serum zinc could be used for diagnosing of zinc deficiency.

In this paper, we described an improved and faster method for analyzing both aluminum and zinc in serum that can be effectively used for the specific, precise, and sensitive determination for essential and toxic metals.

2. Experimental

2. 1. Equipment

For these analyses we used a Model AAnalyst 800 atomic absorption spectrophotometer with a heated graphite furnace and an autosampler 800 (Perkin-Elmer Corp.). Instrument settings and furnace program are detailed in Table 1 for both aluminum and zinc.

Table 1. Modified Thermal Settings for Aluminum/Zinc Heated Graphite Atomizer (HGA-800) Perkin-Elmer AAnalyst 800

		Al /Zn			
Condition	Temp, ^oC	Ramp, s	Hold, s	Internal flow	Gas type
Dry	110/110	1/10	30	250	Normal
	130/150	15/10	30	250	Normal
Char	1500/ 500	10/15	20	250	Normal
Atomize	2300/1800	0	5	0	Normal
Cleaning	2450/2450	1	3/4	250	Normal

Analytical Conditions

Al /Zn	
Wavelength (nm)	: 309.3/213.0
Slit width	: 0.7nm (low) /0.7
Spectral Bandwidth (nm)	: 2.0
Lamp source	: Hollow Cathode Lamp (HCL)
Lamp current	: 25/15 mA
Diluents	: 0.1% Triton X-100
Rinsing solution	: 0.2% HNO_3
Matrix modifier	: No need/ [Mg $(NO_3)_2$]
Background correction	: Zeeman Effect.
Sample volume (μL)	: 20/10

2.2 Regents and Materials

Standards were prepared by appropriate dilution of a 1 mg mL^{-1} (1000 ppm) Certified aluminum and zinc Atomic Absorption Standard (Perkin-Elmer Corp.) with 0.1% Triton X-100. The standards used for aluminum were 10, 20, 40, and 60 ppb respectively and standards used for zinc were 1, 4, 8, 10, and 20ppb respectively.

2. 3. Specimen Collection

Blood serum samples were supplied from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). The specimens were collected into a plastic syringe which is free of aluminum and zinc.

2. 4.Specimen Handling and Precaution

Serum samples were stored in 2.5 mL polypropylene tubes and the dilution were made directly into 2-mL Teflon sampling cups (Perkin-Elmer Corp.) that had been acid-washed for 24 h or longer in 1.6 mol/L HNO_3. The polypropylene tubes, acid-washed Teflon cups, and pipette tips are negligible sources of contamination. All glassware were acid-washed by the method described by Moody and Lindstrom [16], which involves glassware washed in 6 mol L^{-1} HNO_3, and double distilled water.

2. 5. Matrix Modifiers and Standard Solutions

All solutions were prepared in double-dionized water (Barnstead 18.2 Ω cm $^{-}$ resistivity). An aqueous solution of Triton X-100 0f 0.1% (v/v) (Merck) was prepared each working session and used for sample and standard dilution.

Solutions and concentrations tested as chemical modifiers: 0.5, 1, 2 g L^{-1} of aqueous magnesium nitrate Mg $(NO_3)_2.6H_2O$ (Merck suprapure) and also 0.5, 1, 2, 3, g L^{-1} of aqueous Nickle nitrate Ni $(NO_3)_2$.

A 50 µL and a 10 µL aliquot of each specimen of serum were pipetted into separate eppendrof tubes for aluminum and zinc then 950 µL and 990 µL of 0.1% Triton X-100 for aluminum and dionized water for zinc were added. Vortexed well and pooled 900-µL of diluted serum sample into sampling cups. The dilution for aluminum was 20 and for zinc was 100 times. The samples are then analyzed using AAnalyst 800**.**

2. 6. Reference Materials

We pooled 500 µL of SRM into an eppendrof tube and were diluted with 500 µL of 0.1% Triton X-100 solution. This was vortexed well; the dilution factor for SRM was 2. The aliquot was kept for analysis as QC for every 10 samples interval.

2.7. Precaution against Contamination

Volumetric flasks, graduating pipette and auto-sampler cups were cleaned by filling with 20% (v/v) nitric acid and leaving overnight. They were then thoroughly rinsed with distilled water before use. Pipette tips were cleaned before use by repeated pipeting of 20% (v/v) nitric acid and then double distilled water. Rinsing solution for autosampler pipette was 0.2% nitric acid solution.

3. Results and Discussion

3. 1. Optimization of sample dilution and chemical modifier concentration

We modified the method [15-17] of analysis for several reasons and concluded with the following findings.

Optimization of sample dilution: An experiment was carried out for selecting the optimum sample dilution ratio. Triton X-100 was used as the surfactant, most widely used to dilute serum samples. Triton X-100 concentration was chosen selectively not more than 0.25% [21-22] and was found that 0.1% Triton X-100 solution work efficiently for delivering the samples into the graphite tube. These assays were carried out for each chemical modifier and concentration. The study was carried out both for serum samples and aqueous aluminum and zinc standard solution for any significance difference between two matrices and also for comparing the data. In order to do this, serum samples, with two dilution rations, 1/10, 1/50, and 60 µg L^{-1} aqueous aluminum standard solutions and with three dilution ratios, 1/10, 1/50, 1/100 and 20 µg L^{-1} aqueous zinc standard solutions were prepared in 0.1% (v/v) Triton x-100 and assayed. In all cases 20 µL of samples or aqueous standard and 5 µL of chemical modifier were wet-injected into the graphite tube. The main modifications to the instrument default programme were drying and pyrolysis steps. In all steps both peak area and peak height signals were carefully considered. It was found that aluminum with dilution ratio to 1/50 was in good agreement for sensitivity, low background noise than dilution ratio of 1/10 Fig.1. And it was found that for aluminum peak height intensity was in good agreement without any chemical modifiers.

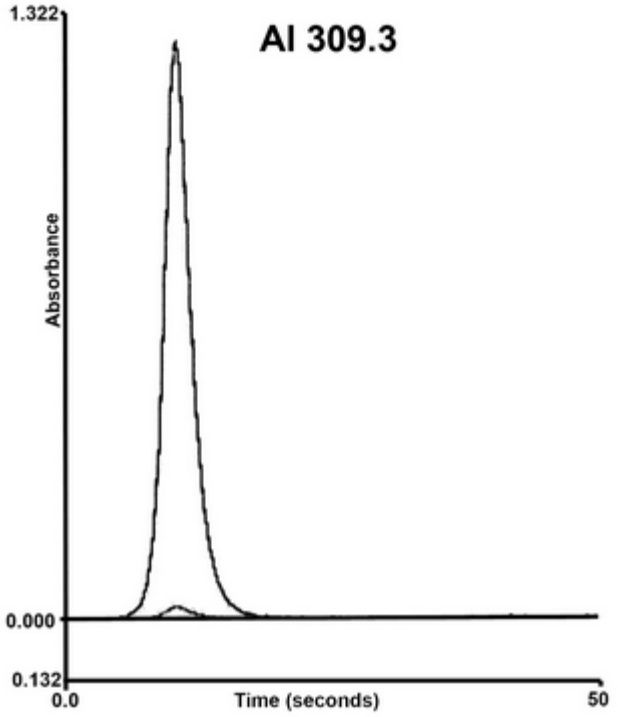

Fig.1. Transient peak of Aluminum

For zinc dilution ratio to 1/100 showed repeatable peak area and peak height signals than dilution ratio of 1/10 and 1/50. Considering dilution ratio 1/100 showed good signal intensities with chemical modifiers [Mg $(NO_3)_2$] rather than both [Mg $(NO_3)_2$] and [Ni $(NO_3)_2$] Fig.2. And the concentration of [Mg $(NO_3)_2$] solution confirmed was 0.1% solution and the volume was 5 µL. Addition of nickel nitrate and lead did not improve the pyrolysis instead it was unpredictable and non-reproducible.

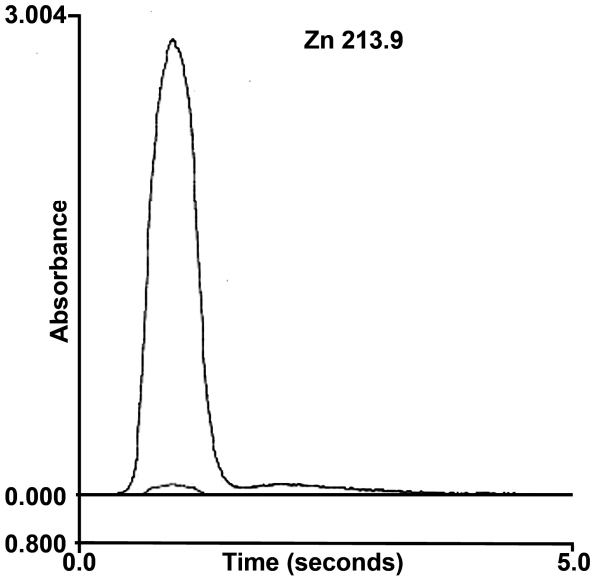

Fig. 2. Transient Peak of Zinc.

3. 2. Furnace Program

First the furnace program described for aluminum by Perkin-Elmer Corp. [17] for use with the transverse heated graphite tube and stabilized temperature platform was modified by us to include an additional drying step before raising the temperature to 1500 ^{o}C. This step was necessary to avoid observed spluttering of the sample during the 1 sec ramp to 130 ^{o}C. A typical 20 μL sample for aluminum was dried with a combined sequence 110 ^{o}C with a ramp of 1 s and a hold time of 20 s, followed by 130 ^{o}C with a 5 s ramp and a hold time of 30 s. Aluminum signal was depressed between 800-1000 ^{o}C but the peak height rises rapidly to plateau, and at ashing temperature above 1600 ^{o}C aluminum is lost by volatilization. We found the best ashing temperature at 1500 ^{o}C rather than recommended 1200 ^{o}C. It was observed that sample volumes greater than 20 μL required longer drying time. A variable volume also affects the spread of solution on the tube platform which effect subsequent atomization. So we maintained a consistent volume for aluminum.

For zinc, sample volume greater than 10 μL required longer drying time. Zinc signal was reproducible at pyrolysis temperature 500 ^{o}C but atomization temperature was best fitted at 1800 ^{o}C Table 1.

The calibration curves showed a linear response up to analyte concentrations of 80 μg L^{-1} for aluminum and 40 μg L^{-1} for zinc with a correlation coefficient of 0.999905/0.999673.

3. 3. Precision

Secondly, the precision of the method was improved by adding a 3-s for aluminum and 4-s for zinc “clean-up” stage at the end analytical cycle. We observed that, when duplicate determination were done for aluminum, the second or third results were always higher than the first, suggesting some carryover. This could be due to zero flow rate used for purge gas during the atomization step, which could allow volatilized aluminum to recondense on the tube surface before being flushed out. We improved the determination of the second and third readings by holding the temperature at 2450 ^{o}C and returning the flow rate of the purge gas to its nominal value of 250 mL/min before the system was finally allowed to cool in order to clean up residual aluminum Many laboratories used 2600 ^{o}C to 2700 ^{o}C for this improvement. We found that temperature higher than 2450 ^{o}C affect the sensitivity and longevity of graphite tube due to deterioration or residue accumulation. The integrity of the tube was evaluated after multiple runs. We found significant loss of signal after 40 to 50 sequential firing of the tube, using in this analysis a 80 μg/L 0.1% Triton X-100 based standard for aluminum and 40 μg/L [Mg $(NO_3)_2$] and 0.1% Triton X-100 based standard for zinc. For zinc an additional experiment was carried out to determine the pyrolysis temperature Fig.3. For zinc, even with the extended drying time,

20 μL of sample was often difficult to dry without sputtering. Initially we injected 15 μL but injection of 10 μL alleviated the problem of spattering.

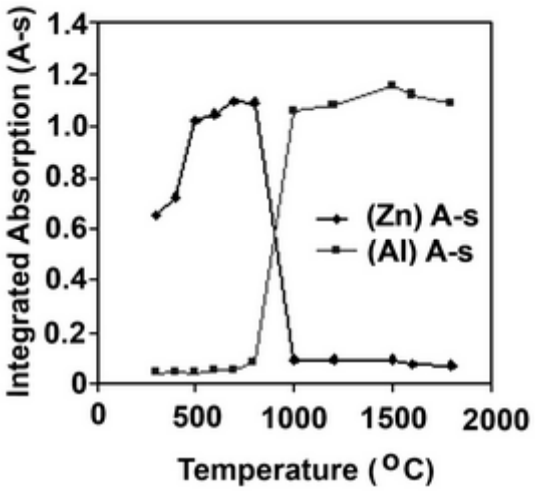

Fig.3. Optimization of the pyrolysis temperature of Aluminum and Zinc

3. 4. One method for two elements

Third advantage of our modification rests in our methods for analysis of both aluminum and zinc using the almost similar protocol for sample preparation. We used 0.1% Triton X-100 instead of matrix modifier for aluminum but for zinc we used both 0.1% Triton X-100 and matrix modifier [Mg $(NO_3)_2$]. Table 1 summarizes the analytical conditions that have been optimized.

Table 2 summarizes the analytical performance of some published methods for serum aluminum and zinc recovery which compare our method favorably. The precision is quite as good as that reported for other methods [18]. Furthermore, these results suggested that quality control can be assured by running standard reference material (SRM) for reliable results.

Table 2. Comparison of Published Methods for Aluminum / Zinc in Serum

Reference no.	Sensitivity	CV, %	Recovery, %
20	50 pg /NA*	5.7/NA	107/NA
18	40 pg /NA	5.3/NA	NA/NA
19	30 pg /NA	2.9 /NA	101/NA
6	24 pg/NA	4.0/NA	95/NA
Present method	11 pg / 2.2 pg	4.9 / 3.18	101/ 97

*NA: Not Analyzed

3. 5. Lower limit detection

Lower detection limits for aluminum detected was 0.016 μg L^{-1} and for zinc was 0.002 μg L^{-1}.

3. 6. Statistical

The mean aluminum concentration for serum samples of 11 healthy subject (age range: 2 yr- to 60 yr) was found to be 36 ± 9.21 μg/L. Mean values for 64 people (2 yr to 60 yr) from poor socio-economic status was 78.60 ± 54.77 μg L^{-1}. Of these subjects, 94% had values of aluminum greater than 64 ± 7.36 μg L^{-1}. The mean zinc values for serum samples of the same 11 healthy subjects were found to be 1.8 ± 0.53 μg mL^{-1} but the zinc level for poor socio-economic ranged from 1.23 ± 0.88 μg mL^{-1}. No additional sample pretreatment was required.

3. 7. Techniques comparison

Finally this paper reported the comparison data of flame and graphite method for analysis of aluminum (Al) and zinc (Zn) Table 3.

Table 3. Comparison of Flame and Graphite method for Aluminum / Zinc Analysis

Elements	Method	Sensitivity	CV%	Reproducibility (%)
Aluminum (Al)	Flame	Not Analyzed	NA	NA
	Graphite Furnace	11 pg	4.90	99.70
Zinc (Zn)	Flame	45 pg	8.11	78.00
	Graphite	2.2 pg	3.18	98.01

It was observed that the sensitivity of aluminum by graphite furnace method is in good agreement but we could not determined aluminum by flame method. Similarly, we tried zinc analysis by both flame and graphite method. It was found that zinc in serum can be determined by flame method and it was comparatively convenient but we found that the SRM results differ greatly from day to day run while the analysis was continued for 2 weeks. We finally analyzed serum zinc by Graphite technique rather than Flame. The sensitivity is increased by 20% one that of Flame technique.

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