Direct Determination of Zinc in Serum by Zeeman Atomic Absorption Spectrometry with a Graphite Furnace

Patrick C. D’Haese,1 Ludwig V. Lamberts,1 Arnold O. Vanheule,2 and Marc E. De Broe1,3

We developed a precise and accurate graphite furnace atomic absorption spectrometric method for the direct determination of zinc in serum. Serum samples are analyzed after 20-fold dilution with water of ultrapure analytical grade. No other reagent is used, from the moment of sampling until measurement. During atomization, the argon flow is kept at 150 mL/min instead of gas stop, to decrease the sensitivity and thus allow lower dilution ratios. Zinc concentrations are determined against a serum-matched calibration curve. Graphite tubes are uncoated and no L’vov platform is used. Between-run CVs were 5.9%, 3.5%, and 1.9% for serum zinc concentrations of 0.93, 1.15, and 1.43 mg/L, respectively. The characteristic mass was 9 pg, and the detection limit (5Δblank + 3SDblank) was 0.060 mg/L.

Additional Keyphrases: calibration · trace elements

1 Department of Nephrology-Hypertension, p/a University Hospital Antwerp, University of Antwerp, Wilrijkstraat 10, B-2650 Edegem/Antwerpen, Belgium.
2 Laboratory of Toxicology, State University of Gent, Belgium.
3 Author for correspondence.

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arbonation steps and (or) the use of a greater number of reagents (8, 11, 13, 14, 16), each of which increases the risk for contamination (17).

The above shortcomings of flame AAS together with the inconvenience or even impossibility of routinely replacing graphite furnaces with burner heads prompted us to develop a graphite furnace AAS method for determining zinc in serum. The proposed contamination-free method involves simple dilution of serum in water. Zinc is determined by direct standardization against serum-matched calibration curves. No modification of the sample matrix, injection system, or atomization source is required.

Materials and Methods

Materials

All items used from the moment of sampling until measurement were considered possible sources of contamination. We avoided the use of glassware and pipettes with metallic bodies. Materials from which detectable amounts of zinc were leached after 24-h contact with water were discarded. We used 50-mL stoppered polypropylene volumetric flasks (Brand GmbH, Wertheim, Germany) and 5- and 10-mL polypropylene test tubes with colorless polypropylene stoppers (Multilab, Zoersel, Belgium). Automatic pipettes used were 50–200 µL and 1.0–5.0 mL Finnpipettes (Labsystems, Helsinki, Finland) with disposable pipette tips (Multilab and Labsystems, respectively). Polyethylene sample cups were prerinsed with one volume of the sample solution before use in the assay.

Reagents. Water used for sample dilution and preparation of standards was purified by the combination of decalcification, de-ionization (VEL, Leuven, Belgium), reverse osmosis (Culligan, Northbrook, IL), and the use of a Milli-Q water purifier (Millipore, Bedford, MA). Water so treated did not contain any detectable zinc (i.e., <0.4 µg/L).

A stock standard of zinc nitrate (J.T. Baker Chemical Co., Phillipsburg, NJ) containing zinc at 1 g/L was used to prepare intermediate and working standards.

Apparatus. Analyzes were performed with a Zeeman 3030 atomic absorption spectrometer (AAS) equipped with an HGA-600 graphite furnace, an AS-60 autosampler, and an Anadex DP-9500B silent scribe, all from Perkin-Elmer Corp., Norwalk, CT.

Methods

Sampling. For venipuncture we used 10-mL sterile syringes (Monovette®, Sarstedt, Nümbrecht, Germany) equipped with 18-gauge syringe needles (Terumo Europe N.V., Baasrode, Belgium). After coagulation, we centrifuged the blood sample and transferred the serum to a 5-mL polypropylene test tube, tight-fitted with a colorless polypropylene stopper, and stored it at 4 or −20 °C. Care was taken to prevent hemolysis.

Sample preparation. Samples were diluted 20-fold with water.

Standard preparation. From the 1 g/L stock standard zinc solution we prepared an intermediate standard of 1 mg/L by diluting 50 µL of the stock standard to 50 mL with water in a polypropylene volumetric flask. This solution was further diluted with water to yield working standards of 0, 20, 40, 60, 80, and 100 µg/L. We added 1.90 mL of each of these solutions to 100 µL of a pooled serum sample with low to normal zinc content to prepare an addition calibration curve, consisting of zinc standards of 0, 0.38, 0.76, 1.14, 1.52, and 1.90 mg/L, respectively.

Flame AAS procedure. Serum zinc concentrations of 34 dialysis patients and of six quality-control specimens obtained with the present procedure were compared with those determined by a flame AAS method (18). In brief, in the latter method, serum was diluted fivefold in 60 mL/L butanol solution. Standards were prepared in the 60 mL/L butanol and were serum-matched for sodium and potassium content. Samples and standards were aspirated into an air/acetylene flame and analyzed with a Model 372 AAS (Perkin-Elmer).

Statistics. We tested equality of regression lines by analysis of variance and covariance with repeated measures (19). To compare zinc concentrations in men and women, we used the unpaired Student's t-test. P<0.05 was considered significant at a two-tailed level.

Results

Time and temperature settings established for the zinc determination in serum by graphite furnace AAS are presented in Table 1. As indicated on the char/atomization curves in Figure 1, the maximal char temperature that could be used without loss of sensitivity was 700 °C. We used an atomization temperature of 2000 °C. Above that temperature, sensitivity did not change but the imprecision (CV) of the determination dramatically increased (Figure 1); on the other hand, atomization temperatures <2000 °C resulted in unacceptable peak broadening. We used ramp atomization (ramp time 1 s), because use of the maximum power heating yielded double peaks.

The influence of the argon flow on both the peak area (A·s) and peak height (A) signals is presented in Figure 2. Use of a gas flow of 150 mL/min allowed us to reduce the dilution ratio 10-fold (from 200-fold to 20-fold), which in turn improved the precision and accuracy of

<table>
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<th>Step</th>
<th>Furnace temp, °C</th>
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| Slit width, 0.7 nm; hollow cathode lamp, 25 mA; purge gas, argon; wavelength, 213.9 nm; injection volume, 5 µL; signal processing, peak area; graphite tubes, uncoated; Zeeman background correction. |
the determination. Increasing the gas flow to >150 mL/min did not significantly affect sensitivity but did result in poorer precision of the analytical result (Figure 2).

We used uncoated graphite tubes. With the proposed time/temperature program, peak broadening was observed in the presence of either pyrolytically coated graphite tubes or the L'vov platform.

Slopes of serum-matched calibration curves differed significantly (P <0.0001) from those for aqueous standards (Figure 3), the equations of the best-fitting curves being \( y = 0.1164x + 0.1014 \) (r = 0.9984) and \( y = 0.1064x + 0.0006 \) (r = 0.9992), respectively. However, calibration curves prepared with serum samples obtained from different subjects (n = 15) did not vary from one another (CV = 2.7%). Provided the peak-area method of quantification is used, addition calibration curves are linear up to zinc additions of 1.90 mg/L (Figure 4). Therefore, samples can be determined by direct standardization against a serum-matched calibration curve.

The detection limit, determined as \( \bar{x}_{\text{blank}} + 3\text{SD}_{\text{blank}} \) was 60 \( \mu \text{g/L} \); the characteristic mass, i.e., amount of analyte yielding a 0.0044 \( A \cdot s \) signal, was 9 pg. The within-run imprecision (measure of repeatability) was 5.25%, 3.85%, and 2.34% (n = 3 runs) for serum zinc concentrations of 0.70, 0.90, and 3.6 mg/L, respectively, whereas the between-run imprecision (measure of reproducibility) was 5.9%, 3.5%, and 1.9% (n = 20 runs) for serum zinc concentrations of 0.93, 1.15, and 1.43 mg/L.

The accuracy of the proposed method is established in Figure 5, correlating our experimental data to the theoretical median zinc values for 14 control serum samples obtained from the external trace-element quality-assessment scheme of the University of Surrey, Guildford, UK. All results fell within the zones of allowable analytical variation (20). Results obtained with the present procedure did not differ from those obtained with a flame AAS method (Figure 6).

The mean ± SD serum zinc concentration for 30 normal subjects (ages 21–55 years) was 1.05 ± 0.16 mg/L and did not differ significantly between men (n = 16) and women (n = 14): 1.07 ± 0.16 vs 1.01 ± 0.15 mg/L.

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Discussion

Several studies on the determination of zinc in biological fluids by flame AAS have been reported since 1965, when Fuwa et al. (21) described the first method for measuring this element. Although flame AAS may provide excellent results, some limitations must be considered. Conventional burner heads and aspiration systems require a 1- to 3-mL sample. Moreover, flame AAS is subject to interferences from solute vaporization, so that one or more preparatory steps are necessary (6, 8, 16). An additional drawback of flame AAS is its limited sensitivity for copper, an element that is often determined simultaneously with zinc for the clinical diagnosis and treatment of several diseases (10). The importance of both elements in dialysis patients as well as in neonates and infants is receiving increased attention (1, 22).

In contrast to flame AAS, sample volumes in graphite furnace AAS are small, i.e., 5 to 100 μL, a prerequisite that is particularly important for pediatric patients and for cases involving frequent blood sampling (23). Moreover, with recent instrumentation such as the Zeeman 3030 AAS, graphite furnaces cannot be replaced by burner heads. Therefore, in circumstances where flame AAS is not a viable option, methods for the determination of zinc by AAS with electrothermal atomization must be developed. Owing to the high sensitivity of the latter technique and the relatively large concentrations of zinc in human serum (mg/L range), most workers have tended to use large dilutions (i.e., 100-fold or more) of samples with graphite furnace AAS (23-25). Although the dilution technique may reduce matrix effects (26) and background absorption, it also reduces precision (27). In addition, even the smallest contamination contributed by the diluting solvent or by the materials used for sample preparation becomes important when high dilution ratios are used (26, 28). In none of the studies involving a 100- to 200-fold dilution were the aqueous and serum-matched calibration curves parallel (23-25). Moreover, failure to obtain linear analytical curves after adding standards to sera with normal zinc content made it necessary for analysts to perform standard additions to serum or serum-matched solutions in which the zinc content had been decreased by laborious dialysis procedures (23, 24) or by treatment with Chelex (25).

In the present study, the dilution ratio could be limited to 20-fold by using uncoated graphite tubes in combination with ramp atomization and an argon flow of 150 mL/min instead of gas stop. Processing signals in peak-area (A · s) instead of peak-height (A) mode allowed us to extend the linear range of the pooled serum calibration curve up to 0.250 A · s, which in turn enabled us to add as much as 1.92 mg of zinc per liter to a serum pool with normal zinc content (i.e., 0.80–1.20 mg/L). Using peak areas instead of peak heights has the added advantage that calibration curves prepared in serum samples are parallel to each other, so that we can use the direct addition calibration technique instead of the time-consuming method of standard additions.

To overcome problems related to curve linearity (or the lack of it), other workers who used a 10- or 20-fold dilution ratio determined zinc in serum by comparison with aqueous calibration curves. However, this latter procedure could be used only after modification of the sample matrix (28, 29), thereby incorporating an increased risk for contamination. To obtain linear calibration curves for zinc determined in 10-fold-diluted serum samples, Levi et al. (27) modified the graphite furnace to significantly decrease the atomic absorption signal or
used an adapted sample-delivery system capable of dispensing microliter and submicroliter sample volumes in the furnace. Both modifications are complicated and cannot be applied in routine laboratories determining trace elements in addition to zinc.

In contrast to others (23, 29) but in accordance with Accominotti et al. (28), the results we obtained by using uncoated graphite furnaces in combination with a 1-s ramp time were superior to those observed in the presence of pyrolytically coated graphite tubes, whether the furnace was equipped with an L’ovv platform or not. The proposed method is accurate and precise as demonstrated by the good correlation between the experimental data and the theoretical median values for zinc in the quality-control serum samples from the quality-assurance survey. The zones of allowable analytical variation chosen by the organizers of the University of Surrey scheme took into account the precision of the available methodologies as well as the clinical importance of certain values (20).

In conclusion, we have developed a simple and rapid method suitable for the routine determination of zinc in serum. A scrupulous selection of instrumental settings (i.e., for time, temperature, gas flow, furnace type, and read-out mode) has resulted in a precise and accurate assay that does not require the use of L’ovv platforms or pyrolytically coated graphite tubes nor does it necessitate modification of the injection/atomization system or the sample matrix. Besides zinc determinations, the procedure for sample preparation offers the possibility for measuring copper and iron (30) in the same diluted sample.

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References