Zinc

Application	Determination in plasma, serum and urine
Analytical principle	Flame atomic absorption spectrometry
Completed in	December 1995

Summary

The method described here is suitable for the determination of zinc in serum, plasma and urine samples of people who have been exposed to zinc at the workplace and those who have not been occupationally exposed to this element.

After dilution of the plasma and serum samples with ultrapure water, or with 1 % HCl in the case of the urine samples, their zinc content is determined by means of flame atomic absorption spectrometry. The quantitative evaluation is carried out using aqueous calibration standards.

Urine

Within-series imprecision:	Standard deviation (rel.)	s_w = 2.4 or 2.5 %
	Prognostic range	u = 5.4 or 5.6 %
	At concentrations of 0.7 or 1.1 mg zinc per litre urine and where n = 10 determinations	
Between-day imprecision:	Standard deviation	s = 4.3 %
	Prognostic range	u = 9.6 %
	At concentrations of 0.7 or 1.1 mg zinc per litre urine and where n = 10 days	
Inaccuracy:	Recovery rate	r = 93–102.6 %
Detection limit:	0.1 mg zinc per litre urine	

Serum

Within-series imprecision:	Standard deviation (rel.)	s_w = 0.9 %
	Prognostic range	u = 2 %
	At a concentration of 1.45 mg zinc per litre serum and where n = 10 determinations	

Inaccuracy:	Recovery rate	r = 96.8–107 %
Detection limit	0.1 mg zinc per litre serum	

Zinc

Zinc (atomic weight 65.4 and atomic number 30) occurs naturally only in the form of compounds. The most important minerals are zinc blende (ZnS), smithsonite ($ZnCO_3$) and galmei (zinc silicate). Zinc is used in many fields of industry. An overview of its uses is presented in Table 1. It has great economic and technical importance in the metal industry for die-casting, for the production of alloys and for the prevention of corrosion (galvanization). In addition, large quantities of the metal are processed to produce electrodes for dry batteries. Due to their antiseptic effect, zinc compounds are used in zinc-containing ointments and powders in medicine.

Table 1. Overview of the chemico-physical properties and industrial uses of zinc and its compounds

Material	Density at 25 °C	Melting point [°C]	Boiling point [°C]	Important industrial uses
Zn	7.14	419.47	907	Hot-dip zinc coating Galvanization Component of alloys Photo printing plates Coating material
ZnO	5.47	1965	–	Pigments Ceramics industry Ingredient of medical tinctures and ointments
$ZnSO_4$	3.74	Decomposition at T > 740 °C	–	Fertilizers Paints Galvanization Ingredients of adhesives and soaps Electrolytic refinement
$ZnCl_2$	2.91	283	732	Organic syntheses Surface coating Dry batteries Wood preservation
ZnS	4.10	1020	–	Monitor screens Oscilloscopes X-ray equipment Paints

Zinc poisoning at the workplace caused by inhalation of finely dispersed zinc oxide has long been known as “metal fume fever”. Besides a general feeling of illness and an

elevated temperature, the symptoms of this acute intoxication include inflammation of the respiratory tract [1, 2].

Zn(II) compounds are relatively innoxious [1, 2]. Acute toxic effects occur when about 150 mg of Zn(II) are ingested orally. Chronic incorporation of Zn(II) leads to gastro-intestinal disorders. In this case, higher zinc values are found in whole blood and in the cellular blood components, and an increased excretion of Zn(II) in urine is also observed. There is still controversy about the clinical symptoms of chronic zinc poisoning. The cases of chronic zinc intoxication described so far may possibly be attributed to the presence of other metallic contaminants (lead, arsenic, antimony, cadmium).

Zinc is one of the essential trace elements. It is a vital component of important enzyme systems. About 40 enzyme systems containing zinc are known. Moreover, zinc functions as a catalytic activator for numerous other enzymes. A summary of the characteristics and biological significance of the zinc metallo-enzymes and metal-enzyme complexes which are activated by Zn(II) can be found in Merian [1], Li [3], Parisi and Vallee [4] and also in Bertelli et al. [5].

Human illness due to zinc deficiency can have various causes. It mainly occurs when alimentary intake is too low, but zinc requirement may increase, e.g. during pregnancy, while breast-feeding or during periods of growth. In addition, increased zinc loss as a result of burns and the intake of chelating agents must be considered. Zinc deficiency is characterized by skin disorders, alopecia, poor healing of wounds, retardation of growth and other clinical symptoms [1, 2].

Intake of zinc can occur via the respiratory or the gastro-intestinal tracts. Ingestion with food is the most important source of zinc intake for people who are not occupationally exposed to the element. In contrast, inhalation intake plays the decisive role at the workplace. In human blood, zinc occurs not only in the serum but also in the cells. The erythrocytes contain 85 % of the zinc content of whole blood, 11 % is bound in the serum, 3 % in the leukocytes and about 1 % in the thrombocytes.

Zn(II) does not occur as a free ion in the blood cells nor in the plasma. It is bound to proteins, mainly albumin. Moreover, binding to haemoglobin is being discussed.

Zinc is mainly excreted through the intestine. Most of the metal which is not absorbed is eliminated with the faeces. Renal excretion is the second most important elimination route. Inorganic zinc is excreted in urine, it is not bound to protein.

Table 2 shows an overview of the zinc concentrations measured by various authors in the serum and plasma of people who have not been occupationally exposed to the element. The consensus range for the zinc concentration in the serum and plasma of healthy adults varies between 800–1200 µg/L [6]. Until recently it was generally assumed that the serum zinc content is about 15 % higher than the plasma content. This assumption is based on the release of zinc from the thrombocytes during the coagulation process. However, recent findings indicate no distinct difference between the zinc concentration in the plasma and the serum provided the serum is separated from the coagulum not more than 30 minutes after the blood sample is withdrawn [7, 8]. In adults no dependence of the values on age or sex has been found [9].

Table 2. Zinc levels in the serum and plasma of persons who have not been occupationally exposed to the element

Reference	N	Range [µg/L]	Mean value [µg/L]	Standard deviation [µg/L]	Method
Davies et al. [10]	–	710 – 1300	950	130	AAS
Hackley et al. [11]	–	–	1180	130	AAS
Farina et al. [12]	10	–	1250	–	AAS
Halsted and Smith [9]	62	720 – 1150	960	130	AAS
Cirla et al. [13]	262	640 – 2200	1030	220	AAS
D'Andrea et al. [14]	24	–	1080	180	AAS
Bruzzone et al. [15]	100	550 – 2960	1760	520	AAS
Fell and Lyon [8]	50	810 – 1140	940	120	AAS

Table 3 shows the zinc concentrations measured by various authors in the urine of people who have not been occupationally exposed to the element. The results show considerable variation. Diet seems to be the primary cause for the variation.

Some investigations of persons who were exposed to zinc at the workplace have been carried out. There are indications that occupational exposure causes an increase in the zinc level in serum, plasma and in urine. Increased zinc concentrations in blood or its various compartments were reported by Cirla et al. [13] and Trevisan [16]. Bruzzone et al. [15] observed an increase in the zinc level in the whole blood and the erythrocytes, but not in the plasma of eight welders. No increase in the zinc level of plasma was determined in numerous other investigations.

Table 3. Zinc concentration in the urine of people not exposed to the element at the workplace [5]

Reference	N	Range [µg/L]	Mean value [µg/L]	Standard deviation [µg/L]	Method
Kahn et al. [17]	30	778 – 1090	517	172	AAS
Farina et al. [12]	80	–	705	273	AAS
El Gazzar et al. [18]	33	–	354	70	Colour
Cirla et al. [13]	218	255 – 1860	765	315	AAS
D'Andrea et al. [14]	24	–	671	388	AAS
Kiilerich et al. [19]	104	41 – 628*	–	–	AAS

* µg/g creatinine

Cirla and his co-workers [13] and Trevisan et al. [16] found increased zinc excretion in the urine of occupationally exposed persons in comparison with a control group. D'Andrea et al. [14] detected enhanced renal zinc elimination in workers in the bronze casting industry, whereas the zinc level in plasma and the erythrocytes remained unchanged.

None of the investigations were able to show a significant relationship between the external exposure to zinc and inner stress. Nevertheless, it can be assumed that the zinc levels in both body fluids are suitable indicators of elevated zinc intake. For the purpose of biomonitoring, determination of zinc excretion in urine may be more suitable than determination of the zinc level in plasma or serum.

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Contents

1 General principles

After dilution of the plasma and serum samples with ultrapure water, or with dilute HCl in the case of the urine sample, the zinc content of the samples is determined by means of flame atomic absorption spectrometry. The quantitative evaluation is carried out using aqueous calibration standards.

2 Equipment, chemicals and solutions

2.1 Equipment

Flame atomic absorption spectrometer with laminar burner and digital display

Zinc mono-element hollow cathode lamp

Centrifuge, at least 3500 g (e.g. from Heraeus)

Sealable, disposable plastic tubes

10, 100, 500 and 1000 mL volumetric flasks

Automatic microlitre pipettes, adjustable between 200 and 1000 µL (e.g. from Eppendorf)

10, 25 and 50 mL graduated pipettes

2.2 Chemicals

Glacial acetic acid

Ultrapure water (ASTM type 1) or double-distilled water

Zinc assay solution 1 g in aqueous solution (e.g. Fixanal® from Riedel-de-Haen)

37 % HCl p.a. (e.g. from Merck)

65 % HNO_3 (e.g. from Merck)

Compressed air

Acetylene

2.3 Solutions

1 M HNO_3 (for cleaning the glassware):
About 400 mL ultrapure water are placed in a 1000 mL volumetric flask. 69 mL of the 65 % HNO_3 are pipetted into the flask. After mixing thoroughly the volumetric flask is filled to the mark with ultrapure water.

1 % HCl solution:
About 300 mL ultrapure water are filled into a 500 mL volumetric flask. Then 16 mL of the 32 % HCl are pipetted into the flask. It is subsequently filled to the mark with ultrapure water.

These solutions can be kept in the refrigerator at 4 °C for an unlimited period.

2.4 Calibration standards

Stock solution 1:
The zinc solution containing 1 g zinc is transferred to a 1000 mL volumetric flask. The flask is subsequently filled to the mark with ultrapure water (1 g/L).
This solution can be stored in the refrigerator at 4 °C for several weeks.

Stock solution 2:
1 mL of stock solution 1 is pipetted into a 100 mL volumetric flask. The flask is subsequently filled to the mark with 1 % HCl (10 mg/L).
This solution can be stored in the refrigerator at 4 °C for several weeks.

Calibration standards containing 0.1 to 0.4 mg zinc per litre are prepared from stock solution 2 by dilution with ultrapure water. These calibration standards can be stored in the refrigerator at 4 °C for at least one week. The standards are prepared according to the following pipetting scheme (Table 4):

Table 4. Pipetting scheme for the preparation of the calibration standards

Volume of stock solution 2 [mL]	Final volume of the calibration standards [mL]	Concentration of the calibration standards [mg/L]
0.1	10	0.1
0.2	10	0.2
0.3	10	0.3
0.4	10	0.4

3 Specimen collection and sample preparation

As in all trace element analyses, reagents of the highest possible purity and thoroughly clean vessels are necessary. Care must also be taken to avoid contamination when specimens are collected. It is important to collect samples after the test persons have changed out of their working clothes. In order to prevent exogenous contamination each of the plastic vessels used for sample collection must be cleansed by leaving them filled with 1 M nitric acid for at least 2 h and subsequently rinsing them thoroughly with ultrapure water before drying them. For determination in the range of the detection limit the cleansing process is further improved by warming the nitric acid.

Urine is collected in plastic bottles at the end of a working shift. The urine samples are acidified with acetic acid (1 mL glacial acetic acid/100 mL urine) and, if they cannot be processed immediately, they are stored in the refrigerator for up to 5 days. If sample processing is further postponed the urine samples must be kept deep-frozen until analysis. For analysis the urine samples are thawed in a water bath at 40 °C and subsequently brought to room temperature. Before an aliquot is taken for analysis the samples are thoroughly shaken to homogenize them.

In the case of analysis of plasma or serum care must be taken to avoid haemolysis of the samples. The blood samples for analysis are slowly withdrawn using either sampling systems suitable for serum preparation (Serum Monovette®, Serum Vacutainer®) or with disposable sampling systems containing an anticoagulant (EDTA-K Monovettes® or EDTA-K Vacutainer®). Preparation of plasma or serum by centrifugation (< 3500 g) must be carried out as soon as possible after withdrawal of the blood sample. The plasma or serum is removed using a pipette and transferred to a sealable plastic tube.

If the sample is not to be analysed immediately it can be stored in the refrigerator (4 °C) for a week. If longer storage is necessary the sample must be kept in the deep-freezer (–18 °C).

For the analytical determination of zinc in the serum and plasma sample 3 mL of the assay material are pipetted into a 10 mL volumetric flask which is subsequently filled to the mark with ultrapure water.

For the analytical determination of zinc in the urine sample 3 mL of the assay material are pipetted into a 10 mL volumetric flask which is subsequently filled to the mark with 1 % HCl.

Each sample series includes at least one reagent blank value in which 1 % HCl is substituted for urine, or ultrapure water for plasma and serum.

4 Operational parameters for atomic absorption spectrometry

Atomic absorption spectrometer:

Wavelength:	213.9 nm
Spectral band width:	1 nm
Spectral slit width:	1 nm
Lamp current:	According to the manufacturer's instructions
Analytical Determination:	Absorption
Flame:	Air/acetylene

5 Analytical determination

For atomic absorption spectrometric measurement the dilute urine, plasma or serum samples are sucked directly out of the 10 mL volumetric flask into the mixture chamber of the burner. After each determination the burner should be rinsed with ultrapure water.

6 Calibration

The aqueous calibration standards (Section 2.4) are analysed by atomic absorption spectrometry as described in Section 4 and 5. A calibration curve is obtained by plotting the absorption of the individual calibration standards as a function of the zinc concentrations used (cf. Figure 1). It is unnecessary to plot a completely new calibration curve for each analytical series. It is sufficient to measure an aqueous calibration standard in every series. The ratio of the value for this standard with the corresponding value in the complete curve is calculated. Each result read off the calibration curve can be adjusted by multiplication with this quotient (or these quotients). A new calibration curve should be plotted if systematic deviation of the quality control results is observed.

The calibration curve is linear between 0.1 and 1.5 mg zinc per litre urine or plasma and serum.

7 Calculation of the analytical result

With the recorded absorptions from the assay samples the equivalent zinc concentration in mg per litre can be read off from the relevant calibration curve. The results are adjusted as described in Section 6. If necessary, a reagent blank value must be taken into account.

The results are multiplied by a factor of 3.33 (because of the 10:3 dilution of the samples) to give the zinc content of the urine, plasma or serum sample.

If the results of the determination are not within the linear range of the calibration curve the samples must be appropriately diluted with ultrapure water (in the case of serum/plasma samples) or 1 % HCl (in the case of urine samples) and analysed anew.

8 Standardization and quality control

Internal statistical quality control of the results is carried out as stipulated in TRgA 410 (Regulation 410 of the German Code on Hazardous Working Materials) [20]. Control urine or serum containing a defined quantity of zinc is measured in each analytical series. Such control materials are commercially available (e.g. from Bio-Rad, "Lyphocheck Urine Metals Control", Munich, as well as control serum and control urine from Nycomed Pharma, Oslo, "Seronorm™ Trace Elements" marketed by Immuno GmbH, Heidelberg, Germany). These materials can also be used to check the accuracy of the method. External quality control can be carried out by participation in round-robin experiments. For the determination of zinc in serum please refer to the "Interlaboratory Comparison Programme" of the Centre de Toxicologic du Quebec, Canada. The analysis of zinc in urine is included in the programme of the "Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V." (German Society for Occupational and Environmental Medicine) [21, 22].

9 Reliability of the method

From the analytical perspective there is no difference between the sample preparation and the quantitative determination of zinc in serum or in plasma. The following reliability criteria for serum samples can be applied to the determination of zinc in plasma with no reservations.

9.1 Precision

Urine

In order to determine the precision in the series defined amounts of zinc were added to aliquots of pooled urine from normal persons to give concentrations of 0.7 or 1.1 mg zinc per litre urine respectively. The urine samples were subsequently analysed 10 times.

The relative standard deviation was calculated as 2.4 or 2.5 % respectively and the equivalent prognostic range was 5.4 or 5.6 % respectively.

In order to determine the precision from day to day the urine samples used above were processed and analysed as described in Section 3 on ten different days. In this case a relative standard deviation of 4.3 % and an equivalent prognostic range of 9.6 % were found.

Serum

In order to determine the precision in the series commercially available control material with a certified zinc content of 1.45 mg per litre serum was analysed ten times.

The relative standard deviation was calculated as 0.9 % and the equivalent prognostic range was 2 %.

9.2 Accuracy

Urine

The accuracy of the method was checked using a commercially available control sample of urine containing a certified content of zinc. According to the manufacturer this sample contained 1.09 mg of zinc per litre urine.

The sample was diluted with 1 % HCl to give zinc concentrations ranging from 0.1 to 1.09 mg per litre urine and the diluted samples were subsequently analysed. Between 93.0 and 102.6 % of the expected value was recovered.

Serum

A commercially available control serum containing a certified amount of zinc was used to check the accuracy of the method. According to the manufacturer this sample had a zinc content of 2.9 mg of zinc per litre serum.

The sample was diluted with ultrapure water to give zinc concentrations ranging from 0.29 to 2.9 mg per litre serum and the diluted samples were subsequently analysed. Between 96.8 and 107 % of the expected value was recovered.

9.3 Detection limit

The detection limit was calculated as three times the standard deviation of the reagent blank value. In this case it was 0.1 mg zinc per litre urine or plasma and serum.

9.4 Sources of error

As in all trace element analyses, it is essential to ensure that the chemicals are of the highest possible purity and that the vessels are thoroughly cleaned. This is especially important for the blood sampling system. Each batch of vessels for the collection of urine and instruments for the withdrawal of blood must be tested for a blank value.

In addition to urine, dilute plasma and serum samples were compared to aqueous calibration standards in this method. When plasma and serum are analysed by flame atomic absorption spectrometry the higher viscosity of these materials must be taken into account. Thus, when the plasma and serum are only slightly diluted, analytical errors can ensue due to the difference in the viscosity between the standard solution and the sample solution. In the zinc analysis described here investigations were carried out to ascertain whether such interference occurred.

The calibration curves were therefore plotted using a commercially available plasma expander (e.g. Haemacell or Macrodex). When the values were compared with the aqueous standards no significantly divergent values were found. Therefore this type of interference obviously does not occur with the plasma and serum dilutions chosen for this method.

In addition, the influence of the urine matrix on the measurements was investigated. For this purpose the absorptions of standard solutions of the same concentration prepared in water or in urine were compared. The results are shown in Table 5.

Table 5. Comparison of the absorptions of standard solutions in water and in urine

Concentration of the calibration standards [mg/L]	Absorption of the aqueous standards	Absorption of the standards in urine
0.10	0.058	0.057
0.20	0.116	0.116
0.30	0.175	0.175
0.40	0.229	0.230

Furthermore, tests were carried out to establish what influence the serum matrix had on the measurement. For this purpose the zinc concentrations in serum samples – obtained when evaluation was carried out on the basis of aqueous standards – were compared with the results obtained when the standards were prepared in serum matrix. The results are presented in Table 6.

Table 6. Determination of the zinc concentrations in serum when evaluation is carried out on the basis of aqueous calibration standards and when standards are prepared in serum matrix

Concentration of the serum samples [mg/L]	Zinc concentration obtained on basis of aqueous standards [mg/L]	Zinc concentration obtained on the basis of standards prepared in serum matrix [mg/L]
0.10	0.10	0.099
0.20	0.20	0.20
0.30	0.30	0.29
0.40	0.40	0.39

For zinc analysis it is essential to ensure that haemolysis has not occurred during (or before) preparation of the plasma and serum. Even when only 1 % haemolysis occurs the zinc level can be expected to rise by about 15 %, as the zinc content in the erythrocytes and thrombocytes is distinctly higher than in the plasma. The results obtained from plasma and serum remain comparable when the serum is separated from the coagulum within almost 30 minutes after withdrawal of the blood samples [7].

10 Discussion of the method

The procedure described here represents a very simple, but analytically reliable method of the determination of zinc in urine and plasma or serum. Investigations carried out by the author and examiner showed that calibration curves obtained using standards prepared in water, or in urine or serum were congruent. Thus, it is unnecessary to use the standard addition procedure.

Moreover, this flame atomic absorption spectrometric method has proved suitable for routine use in occupational medicine because it permits a high sample throughput and it is extremely economical. In contrast, graphite furnace atomic absorption spectrometry has only limited application for routine analysis of zinc in biological materials for occupational medicine [23], because of the high risk of contamination (on account of its greater sensitivity). The use of graphite furnace atomic absorption spectrometry is justified only when small quantities of the sample are available [8]. An overview of further analytical techniques for the quantitative determination of zinc in biological materials can be found in the literature [2].

Instruments used:
Flame atomic absorption spectrometer 420 from Perkin-Elmer

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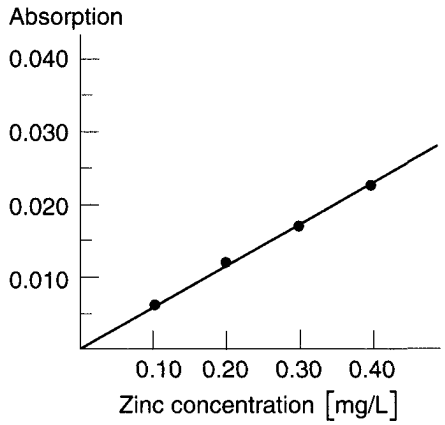

Figure 1. Example of a calibration curve for the determination of zinc