Selenium in serum

Matrix:	Serum or plasma
Hazardous substances:	Selenium and its compounds
Analytical principle:	Graphite furnace AAS
Completed in:	August 2008

Overview of parameters that can be determined with this method and the corresponding hazardous substances:

Hazardous substance	CAS	Parameter	CAS
Selenium	7782-49-2		
Inorganic selenium compounds	–	Selenium	7782-49-2
Organic selenium compounds	–		

Summary

This analytical method allows the quantitative determination of the selenium concentration in serum or plasma using graphite furnace atomic absorption spectrometry with Zeeman background correction. The serum or plasma samples are diluted in a ratio of 1/10 (v:v) with Triton™ X-100 solution and a Pd-Mg matrix modifier and are injected directly into the graphite furnace of the AAS (by use of an autosampler). Quantitative determination is carried out after calibration in matrix, leading to a strictly linear correlation between the measured extinction and the concentration level of selenium.

The method is very sensitive and applicable in routine laboratories with high sample throughput. The detection limit is 3 µg selenium per litre serum, thus selenium can be determined reliably even in the case of extreme selenium deficiency.

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Selenium

Within day precision:	Standard deviation (rel.)	s_w = 3.0% or 2.1%
	Prognostic range	u = 6.7% or 4.7%
	at a concentration of 81 µg or 136 µg selenium per litre serum and where n = 10 determinations	
Day to day precision:	Standard deviation (rel.)	s_w = 4.8% or 4.2%
	Prognostic range	u = 10.0% or 8.8%
	at a concentration of 81 µg or 136 µg selenium per litre serum and where n = 20 determinations	
Accuracy:	Recovery rate (rel.)	r = 97%
	at a nominal concentration of 100 µg selenium per litre serum and where n = 5 determinations	
Detection limit:	3 µg selenium per litre serum	
Quantitation limit:	9 µg selenium per litre serum	

Selenium

No other element has undergone such a dramatic change in its physiological and toxicological assessment over the years as selenium. Up to the 1930s, for example, selenium (Se), like arsenic, was considered to be extremely toxic or carcinogenic [1, 2]. During the 20th century, however, this assessment has changed continuously. Growing evidence for the essentiality of selenium has been reported [3] and in 1973 selenium was described as a part of glutathione peroxidase [4]. Today, selenium is considered to be a trace element of extraordinary importance, especially as essential component in the diet and as a dietary supplement in the prevention and therapy of various diseases, including cancer. This is based on the fact that it is part of specific selenoproteins and/or selenoenzymes, as for example glutathione peroxidase, iodothyronine deiodase, thioreduxin reductase or selenoprotein P [4–7].

Selenium is No. 69 of the elements in the earth's crust and thus belongs to the rarer elements. The burning of fossil fuels and volcanic activity are responsible for the distribution of selenium in the water or the atmosphere. Both, inorganic and organic selenium forms are found in nature. Whereas the inorganic species predominate in the inanimate nature or in industrial processes (e.g. selenite, selenate, elemental selenium, hydrogen selenide), the organic species (e.g. selenomethionine, selenocysteine) predominate in animate nature.

Food consumption is the most important route for human exposure to selenium, whereby the selenium content of foods can vary considerably according to the foodstuffs' origin [8]. The content of selenium in food depends on the selenium content of the soil where plants are grown or animals raised and differs largely [9]. Food of animal origin, in general, contains more selenium than plant based food. In drinking water selenium concentrations are in the low µg/L range. The German Drinking Water Ordinance sets a legal limit of 10 µg selenium per litre drinking water [10].

Occupational exposure to selenium and its inorganic compounds tends to be rare and is described in single cases [11, 12]. Detailed information on the toxicity of selenium and its inorganic compounds is published in the 1999 and 2011 MAK documentations [11, 12]. The maximum workplace concentration (MAK value) for selenium and its inorganic compounds is set at 0.02 mg/m^3 [12].

In addition to the quantification of occupational exposure, the determination of selenium levels is nowadays important to diagnose selenium deficiency. Depending on the objectives, selenium determination can be carried out in blood, urine, serum or plasma. Selenium levels in whole blood allow a statement on the selenium status as a long-term parameter [29]. Although its concentration in urine

Table 1 Concentration range of selenium in serum or plasma in different countries.

Country/Region	Group/Normal values	Reference
Germany	Adults 74–139 µg/L	[13, 14]
	Babies 1–4 months 18–64 µg/L	
	Babies 5–12 months 32–101 µg/L	
	Infants 58–116 µg/L	
	School children 69–121 µg/L	
Germany	Adults 50–120 µg/L	[15]
	Children 0–1 year 33–71 µg/L	
	Children 2–5 years 32–84 µg/L	
	Children 5–10 years 41–74 µg/L	
	Children 10–16 years 40–82 µg/L	
Germany	Children (average age 10.3 years, n = 1918)	[16]
	33–98 µg/L (range),	
	55–72 µg/L (mean values of individual subcollectives)	
Germany	Children 1–5 years (n = 221)	[17]
	74 µg/L (mean value), 41–116 µg/L (5–95th percentile)	
	Children 6–18 years (n = 623)	
	79 µg/L (mean value), 48–116 µg/L (5–95th percentile)	
Canada	Babies 45–104 µg/L (range) (n = 20)	[18]
	Children 1–5 years 99–142 µg/L (range)(n = 20)	
	Children 6–9 years 111–164 µg/L (range) (n = 20)	
	above 10 years and adults 102–205 µg/L (range) (n = 57)	
Iran/Teheran	Women >16 years 67–121 µg/L (5–95th percentile) (n = 24)	[19]
	Men >16 years 79–126 µg/L (5–95th percentile) (n = 106)	
Kuwait	Adults 88 µg/L (mean value) (n = 379)	[20]
USA	Adults >20 years 126 µg/L (mean value) (n = 7497)	[21]
USA	Children 3–11 years 112 µg/L (geometric mean value),	[22]
	93–130 µg/L (10–90th percentile) (n = 1186)	
Japan, Tokyo	Adults 146 µg/L (mean value) (n = 118)	[23]
Taiwan	Adults 111 µg/L (mean value), 41–186 µg/L (range) (n = 2755)	[24]
India	Adults 100 µg/L (mean value), 36–186 µg/L (range) (n = 201)	[25]
Singapore	Adults 122 µg/L (mean value) (n = 244)	[26]
Switzerland	Adults 98 µg/L (mean value), (n = 1847)	[27]
Spain	Adults 111 µg/L (mean value), 60–106 µg/L (range), (n = 150)	[28]

gives no information on the functional selenium status, it reflects the current selenium intake [29]. For this reason an occupational medical determination of selenium levels in urine makes sense. However, most frequently serum or plasma is used for biomonitoring, as the selenium levels in these matrices represent the current selenium status. Moreover, analytical selenium determination in serum or plasma is less problematic and more reliable than in blood or urine. This is why selenium determination in serum was used for deriving a BAT value (*Biologischer Arbeitsstoff-Toleranz-Wert*). This value is set at 150 µg selenium per litre serum [30].

Table 1 shows that selenium concentration in serum or plasma depends greatly on the region where people live. Considerable regional differences even exist within the individual countries [22]. As a rule, children show lower selenium levels than adults [13]. Selenium can be detected both in plasma and in the erythrocytes. Due to the slightly higher selenium content in the erythrocytes the levels in the whole blood are about 1.3 times higher than those found in plasma [31].

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Contents

1 General principles

Selenium is determined using graphite furnace atomic absorption spectrometry with Zeeman background correction. The serum/plasma samples are diluted in a ratio of 1/10 (v:v) with Triton™X-100 solution and a Pd-Mg matrix modifier and are injected directly into the graphite furnace of the AAS spectrometer. Quantitative determination is carried out after calibration in matrix, resulting in a linear relationship between the measured extinction and the spiked concentration levels of selenium. The method is very sensitive and applicable in routine laboratories with high sample throughput. The detection limit is 3 µg Se per litre serum, thus selenium can be determined reliably even in the case of extreme selenium deficiency.

2 Equipment, chemicals and solutions

2.1 Equipment

- Graphite furnace atomic absorption spectrometer with Zeeman background correction and autosampler (e.g. Thermo Elemental)
- Stroke pipettes with adjustable volume between 10–100 µL or 100–1000 µL with suitable pipette tips (e.g. Eppendorf)
- 1000 mL Polyethylene flask with bottletop dispenser (adjustable between 0.5 and 5 mL) (e.g. Dispensette™, Brand)
- 10 mL, 100 mL and 1000 mL Volumetric flasks (e.g. Schott)
- 10 mL Polypropylene vials for autosampler use (e.g. Sarstedt)
- Laboratory shaker (e.g. Reax 2000; Heidolph Instruments GmbH)
- 1.5 mL Sample vials (e.g. Omnilab)
- Lithium-Heparine monovette™ for plasma sampling (e.g. Sarstedt)
- Neutral-S monovette™ for serum sampling (e.g. Sarstedt)

2.2 Chemicals

- Selenium standard solution CertiPUR™; 1000 mg/L Se (e.g. Merck, No. 119796)
- Analytical quality control material (e.g. Seronorm™ Trace Element Serum)
- Nitric acid 65% Suprapur™ (e.g. Merck, No. 100441)
- Magnesium matrix modifier 10 g/L (e.g. Merck, No. 105813)
- Palladium matrix modifier 10 g/L (e.g. Merck, No. 107289)
- Albumin fraction V (from bovine serum) (e.g. Merck, No. 112018)
- Triton™X-100 (e.g. Sigma-Aldrich, No. X100)
- Deionised water

- Argon 5.0 (e.g. Linde)
- Synthetic air (e.g. Linde)

2.3 Solutions

- Dilution solution
  1 mL Triton™X-100 is pipetted into a 1000 mL volumetric flask and dissolved in deionised water. The flask is then made up to the mark with deionised water.
- Matrix modifier solution
  10 mL Pd matrix modifier (10 g/L Pd), 5 mL Mg matrix modifier (10 g/L Mg), 0.8 mL Triton™X-100 and 0.5 mL 65% nitric acid are pipetted into a 100 mL volumetric flask. The flask is then made up to the mark with deionised water.
- Albumin solution
  0.6 g albumin are weighed into a 10 mL volumetric flask and dissolved in deionised water. The flask is then made up to the mark with deionised water.
- Washing solution for the AAS
  100 mL ethanol and 0.5 mL 65% nitric acid are pipetted into a 1000 mL volumetric flask. The flask is made up to the mark with deionised water.

2.4 Calibration standards

- Working solution (1000 µg/L)
  100 µL selenium standard solution (1000 mg selenium/L) and 1 mL 65% nitric acid are pipetted into a 100 mL volumetric flask. The volumetric flask is made up to the mark with deionised water and the solution is homogenised by shaking. The selenium concentration of the working solution is 1000 µg/L.

The working solution should be prepared freshly on a daily basis. Starting with this working solution the calibration standards are prepared according to the pipetting scheme in Table 2, resulting in concentrations up to 240 µg selenium per litre.

Table 2 Pipetting scheme for the preparation of selenium calibration standards.

Volume of working solution (1000 µg/L)	Final volume of calibration standard	Selenium concentration
[µL]	[mL]	[µg/L]
0	10	0
300	10	30
600	10	60
900	10	90
1200	10	120
2400	10	240

To calibrate in matrix, 100 µL calibration standard, 100 µL albumin solution, 600 µL dilution solution and 200 µL matrix modifier solution each are pipetted into a vial.

3 Specimen collection and sample preparation

3.1 Specimen collection

Prior to blood collection, ensure that the blood collection set used is free of selenium. For example, neutral monovettes without addition of coagulation activators are suitable for blood sampling, and lithium-heparine monovettes for plasma sampling. For serum separation the blood sample is allowed to clot for at least 20 min (but no longer than one hour). For plasma separation the blood sample should be mixed thoroughly and centrifuged quickly. After centrifugation (15 min; 2000 × *g*; 15°C), the supernatant is transferred to neutral sample tubes. Haemolysis must be avoided under all circumstances when collecting serum and plasma samples, respectively.

If selenium determination cannot be performed immediately serum or plasma samples may be kept for a few days in the refrigerator at 4°C. For longer storage e.g. for weeks or months the serum or plasma should be deep frozen at –18°C.

3.2 Sample preparation

For sample preparation 700 µL dilution solution, 200 µL matrix modifier solution and 100 µL serum or plasma sample are pipetted into an autosampler vial.

4 Operational parameters

The operational details given below are intended to be a rough guide only and are especially useful if an identical device type is used. In the case of other types, the parameters are to be optimised accordingly. The setting of additional parameters may become necessary when using spectrometers from other manufacturers.

Wavelength:	196.0 nm
Slit width:	0.5 nm
Injection volume:	20 µL
Injection temperature:	80°C
Measuring time:	3 s
Background correction:	Zeeman
Graphite furnace:	pyrolytic-coated
Evaluation:	extinction

Table 3 Tabular presentation of the temperature program (total time 142 s).

Step	1	2	3	4	5	6	7
Temperature [°C]	100	120	600	600	1450	2100	2400
Increase [°C/s]	2	5	50	0	50	0	0
Time (hold) [s]	20	30	30	5	10	3	3
Gas	Ar	Ar	air	Ar	Ar	Ar	Ar
Gas flow [L/min]	0.2	0.2	0.2	0.2	0.2	off	0.2

5 Analytical determination

20 µL of the 1/10 (v:v) diluted serum or plasma sample (see Section 3) are analysed according to the given opperational parameters using atomic absorption spectrometry. The samples are determined in duplicate and the mean values are used for the calculation of the analytical results.

6 Calibration

The measured extinction of each calibration standard is plotted against the corresponding selenium concentration. The calibration graph obtained is linear in the range between 3 and 240 µg selenium per litre serum or plasma. The calibration graph is given in the Appendix (Figure 1).

Recalibration is necessary if the results of quality control show systematic deviations.

7 Calculation of the analytical result

To determine the analyte concentration in a sample, the extinction measured is inserted into the corresponding calibration graph (see Section 6). The selenium concentration level of the sample is obtained in µg/L. Reagent blank values have to be accounted for where necessary. Normally, calculation of the analytical result is performed by the software of the spectrometer.

8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the *Bundesärztekammer* (German Medical Association) [32].

This means that quality control standards are to be included for precision control within each analytical series. For the quantitation of selenium in serum, control material of different manufacturers and also certified reference material is avail-

able. To cover a wide concentration range control material with different concentrations should be used. These should be analysed after calibration, after every twentieth sample and at the end of each analytical series. For external quality control, international quality assessment schemes are available [33, 34].

9 Evaluation of the method

9.1 Precision

To determine the within day precision selenium was analysed ten times in a row using two control materials with nominal values of 81 µg/L and 136 µg/L selenium in serum. The relative standard deviation was determined to be 3.0% and 2.1%, respectively (Table 4). To check the day to day precision selenium was determined in the same control material on twenty different days. The relative standard deviation was determined to be 4.8% (for 81 µg/L Se) and 4.2% (for 136 µg/L Se) (Table 5).

Table 4 Within day precision for the determination of selenium in serum (n = 10).

Analyte	Analyte level [µg/L]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Selenium	81	3.0	6.7
	136	2.1	4.7

Table 5 Day to day precision for the determination of selenium in serum (n = 20).

Analyte	Analyte level [µg/L]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
Selenium	81	4.8	10.0
	136	4.2	8.8

9.2 Accuracy

Accuracy was determined using internal and external quality assurance. For internal quality assurance "Seronorm™ Trace Elements Serum" Level 1 and Level 2 control materials (Sero, Norway) were analysed. The mean values (n = 20) of the day to day precision were 82 and 140 µg/L and are in line with the nominal values of 81 and 136 µg/L.

The quantitation of a spiked serum sample (60 µg/L selenium spiked to a physiological concentration of 40 µg/L) led to a recovery rate of 97%.

For external quality assessment the German External Quality Assessement Scheme (G-EQUAS) No. 39 for Analyses in Biological Materials, carried out by the

Institut und Poliklinik für Arbeits-, Sozial- und Umweltmedizin (Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine) at the University of Erlangen-Nuremberg was participated in [33]. Using the described method selenium concentrations of 79 and 129 µg/L (nominal values: 75 and 133 µg/L) were determined in two plasma samples. Participation in a british external quality assessment scheme [34] also yielded good results. 24, 88 and 157 µg/L were determined in the control materials 859, 860 and 861 from October 2007 (nominal values: 23, 87 and 150 µg/L).

Table 6 Quality assessment for the determination of selenium in serum.

Control material	Analysed value [µg/L]	Nominal value [µg/L]	Recovery rate *r* [%]
Seronorm™	82	81	101
Trace Elements serum	140	136	103
Spiked serum	97	100	97
G-EQUAS No. 39	79	75	105
	129	133	97
University of Surrey	24	23	104
859, 860 and 861	88	87	101
	157	150	105

9.3 Detection limit and quantitation limit

A detection limit of 3 µg selenium per litre serum or plasma was estimated for the given procedure. This correspond to a detection limit of 0.3 µg selenium per litre in the measured solution. The detection limit was determined according to the 3 s criterion based on the sensitivity and the standard deviation of the extinction of a blank value solution (n = 10). This corresponds to a quantitation limit of 9 µg/L.

9.4 Sources of error

To avoid interferences by the spectral background the use of Zeeman background correction is required. In addition the temperature program must be carefully optimised in order to minimise background signal during atomisation. To avoid selenium losses prior to the atomisation step a suitable matrix modifier must be selected. The Pd-Mg modifier has proved its value. Interferences from residues in the form of carbon particles in the graphite furnace can be avoided by carrying out ashing with synthetic air. For this reason the ashing procedure is performed in two individual steps (Table 3).

Variations in sensitivity, due to aging of the graphite furnace (e.g. during an extended run), can be compensated for by a dynamic measurement value correc-

tion as an additional quality control measure. For this a control standard and a blank value are analysed after every tenth sample and are included in the calculation of the analytical result. This kind of standardisation can be configured in the software of modern spectrometers.

Using graphite furnace AAS no transport interferences are observed. Nevertheless, the pipetting accuracy of the autosampler is affected negatively, when all pipetting and dilution steps are carried out by the autosampler itself (pipetting of the samples, addition of the modifier and of the Triton-X-100 solution). In the case of serum, plasma or blood, a thin film can form on the surface of the autosampler capillary leading to negative effects on pipetting accuracy. Therefore, the author of this method diluted the samples and calibration standards manually to ensure better long-term stability within large analytical series.

The risk of selenium contamination from reagents, equipment or ambient air must be considered at all times. Reagents used must be tested for purity at regular intervals. Rigid purity standards have to be applied for the used equipment, such as vials, volumetric flasks, tubes and pipettes. Clean room conditions are not necessary but useful.

10 Discussion of the method

The method provides accurate and reliable results for selenium levels in serum or plasma using graphite furnace atomic absorption spectrometry with Zeeman background correction. The method is selective, sensitive, easy to handle and therefore suitable for laboratories with a high sample throughput. Due to the high sensitivity of graphite furnace technique, determination is also possible within the concentration range of extreme selenium deficiency in humans. Selenium levels after occupational exposure or intoxication can also be determined by this method. For these determinations the samples must be further diluted if necessary.

For method validation, calibration was carried out using an albumin solution as matrix, because this behaves very similar to serum in the graphite furnace AAS. This procedure was used, as the performance of a standard addition for each individual sample would be a very time consuming process.

As a third calibration strategy one could perform standard additions on the same sample and convert this standard addition calibration into an external calibration for the analysis of the following samples. However, the disadvantage of this calibration procedure is that the selenium levels of a number of real samples would be below the lowest calibration standard.

As selenium also forms a gaseous hydride, hydride-AAS might be used alternatively, ensuring even lower detection limits. However, in a clinical laboratory dealing with a large number of samples, the simple and fast graphite furnace technique should be preferred. Sensitivity, selectivity and robustness are fully sufficient. Finally, compared with the hydride technique, the graphite furnace techni-

que requires less sample material, avoids some additional reagents for digestion and reduction of selenium and is less time-consuming.

Instruments used:

- Thermo Elemental SOLAAR M6 series AA spectrometer with FS95 autosampler and Zeeman background correction (Thermo Elemental)

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12 Appendix

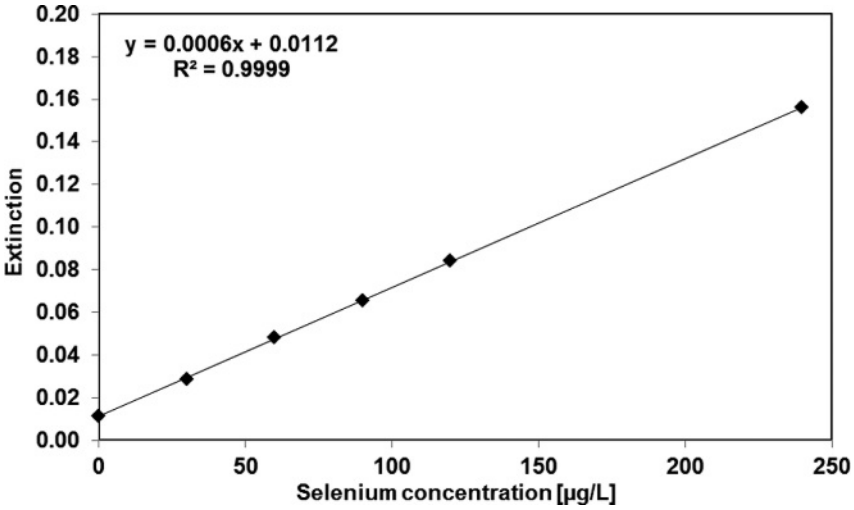

Fig. 1 Calibration graph for the determination of selenium in serum.