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Selenium and its compounds – Determination of total selenium in urine by ICP-MS

Biomonitoring Method – Translation of the German version from 2023

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Abstract

The working group "Analyses in Biological Materials" of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area developed and verified the presented biomonitoring method.

Selenium is used in different industrial processes. In the workplace, employees are mainly exposed to elementary selenium and inorganic selenium compounds. In contrast, the non-occupationally exposed general population ingests mainly organic selenium compounds, like selenomethionine and selenocysteine, via the diet. Following exposure, selenium can be determined in serum/plasma and in whole blood/erythrocytes. While the selenium concentrations in these matrices reflect exposure from recent weeks or even months, urinary selenium concentrations are suitable for the determination of short-term exposure (e.g. of the last shift).

The aim of this work was to develop a selective method for the determination of total selenium in urine while avoiding the selenium enhancement effect caused by volatile selenium species (e.g. dimethyl selenide or dimethyl diselenide). The method has been comprehensively verified, and the reliability data have been confirmed by replication and verification of the procedure in a second, independent laboratory.

Urine samples are mineralised by microwave-assisted digestion using an acidic hydrogen peroxide-containing solution, thereby converting the various selenium species present in the urine into selenite. The selenium concentrations in the diluted digestion solutions are determined by mass spectrometry with inductively coupled plasma (ICP-MS) on $m/z = 78$. Germanium is used as internal standard.

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The microwave-assisted digestion allows for the accurate quantification of total selenium in urine even when volatile selenium species are present in the samples. The good precision and accuracy data show that the method provides reliable and accurate measurement values. The method is both selective and sensitive, and the quantitation limit of 0.5 µg/l urine is sufficient to determine occupational as well as background exposure to selenium and its compounds.

1 Characteristics of the method

Matrix	Urine
Analytical principle	Inductively coupled plasma-mass spectrometry (ICP-MS)

Parameter and corresponding hazardous substances

Hazardous substance	CAS No.	Parameter	CAS No.
Selenium	7782-49-2	Selenium	7782-49-2
Selenium dioxide	7446-08-4		
Selenium trioxide	13768-86-0		
Selenate	14124-68-6		
Selenite	14124-67-5		
Selenium hydride	7783-07-5		

Reliability criteria

Selenium

Within-day precision:	Standard deviation (rel.) Prognostic range at a spiked concentration of 41.9 µg or 327 µg selenium per litre of urine and n = 8 determinations	s_w = 2.2% or 1.0% u = 5.2% or 2.4%
Day-to-day precision:	Standard deviation (rel.) Prognostic range at a spiked concentration of 41.9 µg or 327 µg selenium per litre of urine and n = 6 determinations	s_w = 5.8% or 5.3% u = 14.9% or 13.4%
Accuracy:	Recovery rate (rel.) at a nominal concentration of 8 µg, 41.9 µg, 230 µg, or 327 µg selenium per litre of urine and n = 3 determinations	r = 91%, 101%, 86%, or 102%
Detection limit:	0.2 µg selenium per litre of urine	
Quantitation limit:	0.5 µg selenium per litre of urine	

2 General information on selenium and its compounds

Selenium (Se; relative atomic mass: 78.97; atomic number: 34) is a metalloid in the sixth group of the periodic table of the elements and occurs as the natural isotopes ^{74}Se (0.9%), ^{76}Se (9.0%), ^{77}Se (7.6%), ^{78}Se (23.5%), ^{80}Se (49.8%), and ^{82}Se (9.2%). Pure selenium minerals are very rare; in contrast, selenides are often found in small amounts accompanied by isomorphic sulfides. In this way, it occurs in iron sulfides, sphalerite, and chalcopyrite and, when these ores are roasted, accumulates as selenium dioxide in flue ash. Anode sludge from electrolytic copper refinery is a primary source for

selenium (RÖMPP-Redaktion and Hartwig 2006). The most prominent selenium producers are China, Japan, Germany, Belgium, and Russia, whereby worldwide production for the year 2020 was around 3300 t (Austrian Federal Ministry of Finance 2022).

Selenium is a very important component in industrial processes and is primarily used in the glass, ceramics, metal, and chemicals industries as well as in electrical engineering. In these areas, it is used for the dyeing and bleaching of glass, the manufacture of alloys, the vulcanisation of rubber, the manufacture of fertilisers and pigments as well as in the production of solar cells (Langner 2000; RÖMPP-Redaktion and Hartwig 2006).

Selenium is an essential trace element for humans. In the form of selenocysteine, it is incorporated into the structure of more than 25 different enzymes such as glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases (Lu and Holmgren 2009). As such, selenium plays a major role in a number of physiological processes such as protection against oxidative stress, redox-regulated signalling pathways, and the synthesis of thyroid hormones (Rayman 2000, 2012). Aside from occupational exposure, selenium intake occurs primarily via the diet, whereby a daily intake of 70 µg and 60 µg is recommended for men and women, respectively (D-A-CH 2016). The tolerable upper intake level for selenium lies at 300 µg per day (SCF and NDA 2006).

Employees in the workplace are primarily exposed to elementary selenium and inorganic selenium compounds, whereby the selenium compounds are primarily absorbed in the form of water-soluble inorganic compounds via oral, inhalation, and dermal routes. In contrast, the non-occupationally exposed general population ingests mainly organic selenium compounds, like selenomethionine and selenocysteine, via the diet (WHO 1987). The oral absorption rates for inorganic selenites and selenates range from 62–84% and 92–94%, respectively, and those for organic selenium compounds from 75–95% (Rettenmeier 2019). The metabolism of selenium and its compounds is comprehensively described in the methods on the determination of various selenium species published by the Commission (Hildebrand et al. 2022 a, b; Jäger et al. 2022).

Selenium is eliminated biphasically with average half-lives of 2.4 ± 0.3 and 162 ± 9 days. Inorganic selenites or selenates are excreted more rapidly than organic selenium compounds (such as selenomethionine), which can be explained by the incorporation of selenomethionine in proteins (RKI 2006).

The evaluation of essential selenium supply as well as any excessive selenium exposure is carried out by examining selenium levels in serum or plasma (short-term status) or in whole blood or erythrocytes (long-term status) (Rettenmeier 2019). These matrices can represent selenium intake from recent weeks or even months, but not short-term exposure in the past few hours. Due to its rapid increase after exposure, urinary selenium concentration is suitable for the determination of such short-term exposure (Göen and Greiner 2018; Greiner et al. 2020).

Representative selenium concentrations determined in the urine of the non-occupationally exposed general population, both within Germany and abroad, are presented in Tables 1 and 2. Data on renal selenium excretion following occupational exposure are listed in Table 3.

Tab. 1 Selenium concentrations determined in the urine of the non-occupationally exposed general population of Germany

Collective (number of persons n)	Mean ± SD (range)	Reference
Adults (n = 18)	16.0 ± 4.6 µg/l (9–23 µg/l)	Schierling et al. 1982
Adults (n = 24)	14.8 ± 6.9 µg/l (3.2–26.3 µg/l) 13.0 ± 3.8 µg/g creatinine (6.3–20.0 µg/g creatinine)	Oster and Prellwitz 1990
Adults (n = 87)	14 µg/l (3–60 µg/l)	Heitland and Köster 2006
Adults (n = 47)	10.4 µg/l (3.5–39.6 µg/l)[a)] 15.7 µg/g creatinine (8.5–39.1 µg/g creatinine)[a)]	Jäger et al. 2013
Adults (n = 102)	14.1 µg/l (1.8–52 µg/l)	Heitland and Köster 2021

[a)] Median (range)

Tab. 2 Selenium concentrations determined in the urine of the non-occupationally exposed general population of other countries

Country	Collective (number of persons n)	Geometric mean (95th percentile)	References
Belgium	Adults (n = 1001)	25.1 µg/l (61.6 µg/l)	Hoet et al. 2013
Canada	Children and adults (6–79 years; n = 5738)	51 µg/l (130 µg/l)	Health Canada 2013
Slovenia	Adults (n = 812)	13.5 µg/l (0.50–121 µg/l)[a)]	Snoj Tratnik et al. 2019
United Kingdom	Adults (n = 132)	13.4 µg/l (33.4 µg/l)[b)]	Morton et al. 2014

[a)] Geometric mean (range)
[b)] Median = calculated value (95th percentile)

Tab. 3 Selenium concentrations in urine following occupational exposure

Collective (number of persons n)	Sample matrix	Mean ± SD (range)		References
		Workers	Controls	
Selenium-rectifier manufacture, England (1517 samples from 200–300 workers; 793 controls)	Urine	84 µg/l (20–4900 µg/l)	34 ± 24 µg/l (0–150 µg/l)	Glover 1967
Selenium-processing plant, Germany (20; 20 controls)	Urine (post-shift)	107 µg/g creatinine (16–816 µg/g creatinine)[a)]	23 µg/g creatinine (12–50 µg/g creatinine)[a)]	Göen et al. 2015
Copper refinery, Canada (20; 20 controls)	Urine	92.9 ± 42.8 µg/l (34.0–190 µg/l)	74.6 ± 25.3 µg/l (26.7–118 µg/l)	Rajotte et al. 1996
Steel production, Taiwan (23; 23 controls)	Urine	67.7 ± 27.4 µg/l (24.1–114 µg/l)	33.2 ± 12.9 µg/l (13.0–58.9 µg/l)	Horng et al. 1999
Selenium-processing plant, Germany (14; 18 controls)	Urine (pre-shift)	50.6 µg/g creatinine (20.7–253 µg/g creatinine)[a)]	18.7 µg/g creatinine (9.20–40.6 µg/g creatinine)[a)]	Greiner et al. 2020
	Urine (post-shift)	71.8 µg/g creatinine (22.1–340 µg/g creatinine)[a)]		

[a)] Median (range)

For selenium and its inorganic compounds, the Commission has derived a BAT value (*Biologischer Arbeitsstoff-Toleranz-Wert*; biological tolerance value) of 150 µg selenium/l plasma and a MAK value (*maximale Arbeitsplatz-Konzentration*; maximum workplace concentration) of 0.02 mg selenium/m^3 I (inhalable fraction, as selenium). In addition, selenium and its inorganic compounds have been classified as Category 3 carcinogens and are designated with an "H" (danger from percutaneous absorption). Details on the toxicological evaluation can be found in the corresponding MAK documentations published by the Commission (DFG 2022; Hartwig 2014, 2015; Rettenmeier 2019). Furthermore, the Commission has derived a BAR (*Biologischer Arbeitsstoff-Referenzwert*; biological reference value) of 30 µg selenium/g creatinine for the urine matrix (Greiner et al. 2021). Only German and Western European studies were used to derive this BAR, as the varying selenium concentrations in soil substantially influence the selenium concentrations in food products and, in turn, selenium intake (Combs 2001).

Due to the varying toxicological potential of absorbed or metabolically formed selenium compounds (Nuttall 2006), an analytical differentiation of the selenium species found in urine is imperative for certain evaluations of occupational and non-occupational exposure. For this reason, the Commission developed and published three methods for the urinary determination of selenosugar 1, selenosugar 2, selenomethionine, selenoethionine, methylselenic acid, *Se*-methylselenocysteine, and *Se*-methylselenoglutathione (Hildebrand et al. 2022 a); selenite and selenate (Jäger et al. 2022); as well as selenosugar 3 and trimethylselenonium (Hildebrand et al. 2022 b).

3 General principles

The analytical method described in the following sections serves for the quantitative determination of total selenium in urine, whereby the low quantitation limit allows for the quantification even of very low urinary selenium concentrations.

In order to avoid systematic errors by volatile selenium components in actual samples as well as for improved matrix adjustment, digestion is performed as part of sample preparation. For this purpose, the urine samples are diluted with the hydrogen peroxide-containing, acidic digestion solution and subsequently mineralised by microwave-assisted pressure digestion. The various selenium species present in urine are thereby converted into inorganic selenium, according to Wang et al. (2001), into selenite.

The selenium concentrations of samples thus processed are determined by ICP-MS in reference to the selected isotope ^{78}Se. Calibration is performed using matrix-matched standards which are processed and measured analogously to the samples. Germanium is added to the samples as an internal standard.

4 Equipment, chemicals, and solutions

4.1 Equipment

- Mass spectrometer with inductively coupled plasma and octopole reaction cell (e.g. Agilent 7500cx, Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany)
- ICP-MS autosampler (e.g. Agilent ASX-500 Series ICP-MS autosampler, Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany)
- Microwave-assisted digestion system (e.g. Multiwave 3000 with 8SXF100 rotor, Anton Paar Germany GmbH, Ostfildern-Scharnhausen, Germany)
- 100-ml polytetrafluoroethylene decomposition vessels (e.g. Anton Paar Germany GmbH, Ostfildern-Scharnhausen, Germany)
- Vortex mixer (e.g. ROTILABO® Mini Vortex, Carl Roth GmbH + Co. KG, Karlsruhe, Germany)
- 13-ml and 50-ml polypropylene tubes (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)
- 125-ml screw-top polypropylene sample containers (e.g. VWR International GmbH, Darmstadt, Germany)
- 10-ml polypropylene volumetric flasks (e.g. VWR International GmbH, Darmstadt, Germany)
- 100-ml polypropylene graduated cylinders (e.g. VWR International GmbH, Darmstadt, Germany)
- Various piston-stroke pipettes (e.g. Eppendorf AG, Hamburg, Germany)
- Urine cups (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)
- Urine Monovettes® (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)

4.2 Chemicals

Unless otherwise specified, all chemicals must be a minimum of *pro analysi* grade.

- Selenium ICP standard 1000 mg/l, traceable to SRM from NIST, SeO_2 in HNO_3 2–3%, Certipur® (e.g. No. 1703500, Merck KGaA, Darmstadt, Germany)
- Germanium ICP standard 1000 mg/l, traceable to SRM from NIST, $(NH_4)_2GeF_6$ in H_2O, Certipur® (e.g. No. 1703200, Merck KGaA, Darmstadt, Germany)
- Nitric acid 65%, Suprapur® (e.g. No. 100441, Merck KGaA, Darmstadt, Germany)

- Hydrochloric acid 30%, Suprapur® (e.g. No. 100318, Merck KGaA, Darmstadt, Germany)
- Hydrogen peroxide 30%, Suprapur® (e.g. No. 107298, Merck KGaA, Darmstadt, Germany)
- Ultra-pure water (e.g. Milli-Q® Direct Water Purification System, Merck KGaA, Darmstadt, Germany)
- Argon 4.6 (e.g. Linde AG, Pullach, Germany)
- Hydrogen N50 (e.g. AIR LIQUIDE Deutschland GmbH, Leipzig, Germany)

4.3 Solutions

- Nitric acid (3%)

  95 ml of ultra-pure water are placed in a 125-ml sample container and 4.6 ml of 65% nitric acid are carefully added. The vessel is sealed and the solution is mixed by shaking.

The 3% nitric acid is stored in the refrigerator at 4–6 °C.

4.4 Internal standard (ISTD)

- ISTD spiking solution (500 µg/l)

  About 200 ml of ultra-pure water are placed in a 250-ml volumetric flask. 125 µl of the germanium ICP standard (1000 mg/l) are then added by pipetting. The volumetric flask is made up to the mark with ultra-pure water.

The germanium ISTD spiking solution can be stored in the refrigerator at 4–6 °C.

4.5 Calibration standards

- Spiking solution I (10 mg/l)

  100 µl of the selenium ICP standard (1000 mg/l) are pipetted into a 10-ml volumetric flask, which is then made up to the mark with ultra-pure water.

- Spiking solution II (100 µg/l)

  100 µl of spiking solution I are pipetted into a 10-ml volumetric flask, which is then made up to the mark with ultra-pure water.

The selenium spiking solutions can be stored in the refrigerator at 4–6 °C.

The calibration standards are prepared in pooled urine from persons without occupational selenium exposure in a concentration range of 0.5–350 µg Se/l. For this purpose, the spiking solutions are pipetted into 10-ml volumetric flasks according to the pipetting scheme given in Table 4. The flasks are then made up to the mark with pooled urine. The calibration solutions are processed analogously to the urine samples as described in Section 5 and 6.

Tab. 4 Pipetting scheme for the preparation of calibration standards for the determination of total selenium in urine

Calibration standard	Spiking solution I [µl]	Spiking solution II [µl]	Pooled urine [ml]	Analyte concentration [µg/l]
0	–	0		0
1	–	50		0.5
2	–	100		1
3	–	500		5
4	25	–	ad 10	25
5	50	–		50
6	100	–		100
7	250	–		250
8	350	–		350

5 Specimen collection and sample preparation

5.1 Specimen collection

The urine samples are collected in sealable urine cups. The urine samples of occupationally exposed workers are collected at the end of a shift; in cases of long-term exposure, samples are collected after several shifts. If the samples are not measured immediately, they are drawn into Urine Monovettes® and stored in the freezer at –18 °C until processing.

5.2 Sample preparation

The urine samples are brought to room temperature and mixed on the vortex mixer. 5 ml of the urine sample are transferred into a 100-ml decomposition vessel. Subsequently, 1.5 ml of ultra-pure water, 1.0 ml of 65% nitric acid, 0.5 ml of 30% hydrochloric acid, 2.0 ml of 30% hydrogen peroxide, and 0.5 ml of the ISTD spiking solution are added by pipetting. The decomposition vessel is sealed and the preparation is mixed on the vortex mixer. The preparation is then mineralised in the microwave-assisted digestion system at 1400 W for 30 min. After digestion, the preparation is allowed to cool to room temperature and is then transferred into a 50-ml polypropylene tube. The preparation is made up to 20 ml with ultra-pure water, and again mixed on the vortex mixer for 15 seconds. Of the digestion solution thus diluted, 3 ml are transferred into a 13-ml polypropylene tube and analysed as described in Section 6.

6 Operational parameters

Analytical determination is performed by ICP-MS.

ICP-MS parameter

Nebuliser:	MicroMist
Spray chamber:	Scott, quartz
Spray-chamber temperature:	10 °C
Rf power:	1500 W
Sampler/skimmer cone:	Nickel
Carrier gas:	0.9 l argon/min

Makeup gas:	0.2 l argon/min
Reaction gas:	3.5 ml hydrogen/min
Number of measurements per mass trace:	3
Measurement mode:	Time-resolved analysis
Selected isotopes:	^{78}Se (analyte); ^{72}Ge (ISTD)
Measurement time per mass trace:	^{78}Se: 1.0 s; ^{72}Ge: 0.5 s

The instrument-specific parameters must be determined and adjusted by the user for the specific ICP-MS system used. The parameters indicated in this section have been ascertained and optimised for the device configuration used during method development. In general, the ICP-MS system must undergo the daily optimisation routine as directed and achieve the expected specification values, which may vary depending on the manufacturer. Other nebulisers may also be used for sample introduction.

7 Analytical determination

The determination of selenium concentration in the urine samples is carried out by measuring the signal intensity of the isotope ^{78}Se. Due to possible interference from clusters of the same mass/charge ratio, the isotope ^{80}Se is not used for the quantification of selenium within the framework of this method (see Section 11.5). The measurements for this method are carried out in reaction-cell mode using hydrogen as the reaction gas. This approach avoids disturbances from polyatomic interferences, such as $Ar^{40}Ar^{38}$ clusters, when using the isotope ^{78}Se.

In addition to the samples to be analysed, the calibration standards, and the quality-control materials, a reagent blank consisting of ultra-pure water is included as part of each analytical run.

After each measurement, the system should be rinsed with diluted nitric acid (3%) for 60 s to avoid any potential memory effects.

8 Calibration

The calibration standards (see Section 4.5) are prepared analogously to the urine samples (see Section 5.2) and analysed. The calibration curve is obtained by plotting the quotients of the measurement signals of the analyte and the ISTD against the corresponding concentration of the calibration standard. The calibration curve for the determination of selenium is linear from 0.5–350 µg/l under the described analytical conditions.

Figure 1 shows a representative calibration curve for the determination of total selenium in urine.

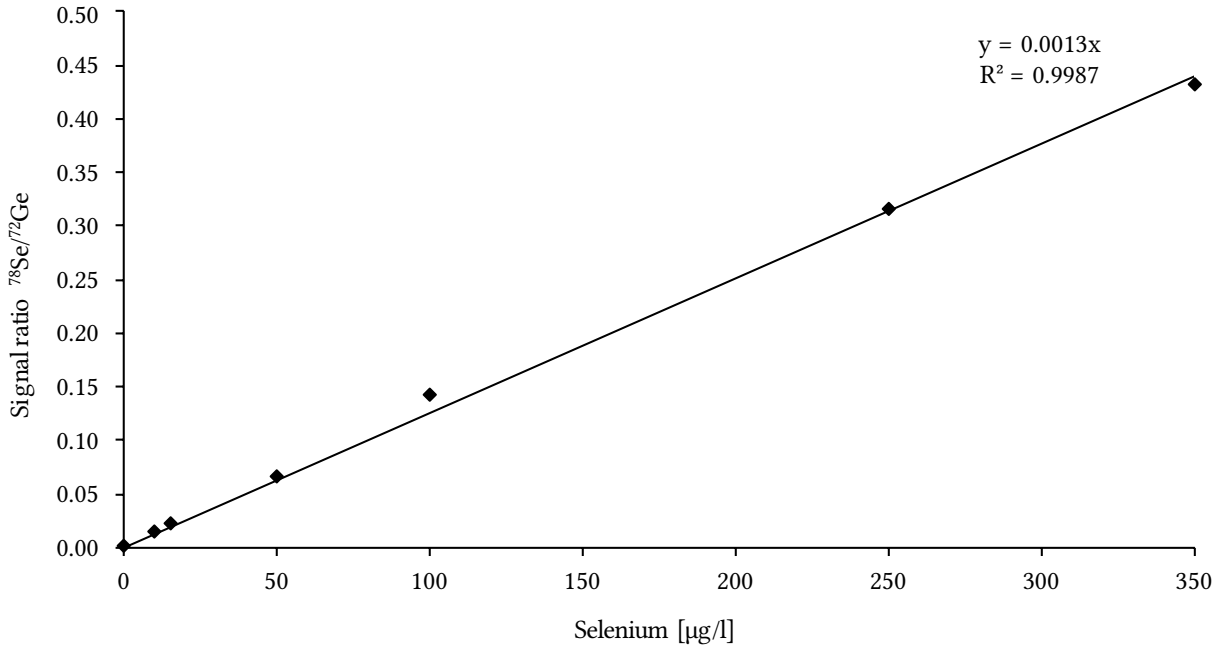

Fig. 1 Calibration curve for the determination of total selenium in urine

9 Calculation of the analytical results

The analyte concentration of a sample in µg/l can be calculated by entering the quotient of the analyte and ISTD measurement signals into the calibration function for the corresponding analytical run. The calibration range must be adjusted to the expected range of selenium concentrations. If the measured result lies above the calibration range, the sample in question is diluted with ultra-pure water, reprocessed, and newly analysed.

10 Standardisation and quality control

Quality assurance of the analytical results is carried out as stipulated in the guidelines of the *Bundesärztekammer* (German Medical Association) and in a general chapter published by the Commission (Bader et al. 2010; Bundesärztekammer 2014).

For quality assurance, two quality-control samples with different analyte concentrations are processed and analysed together with the samples as part of each analytical run. The selenium concentration of the control materials should thereby be selected to guarantee correct measurement across the entire working range. The nominal value and tolerance range (mean ± three standard deviations) of the quality-control materials are ascertained in a pre-analytical period by measuring the control materials on ten different days (Bader et al. 2010).

11 Evaluation of the method

The reliability of this method was confirmed by comprehensive validation as well as by replication and verification in a second, independent laboratory.

11.1 Precision

To determine the precision data, G-EQUAS interlaboratory-comparison materials (48/2A or 48/2B) for ranges relevant for both environmental and occupational medicine were used.

Within-day precision

For the determination of within-day precision, each of the materials of the G-EQUAS interlaboratory trial 48/2 were processed and subsequently analysed eight times in one day. The within-day precision data are presented in Table 5.

Tab. 5 Within-day precision for the determination of total selenium in urine (n = 8)

Material	Nominal concentration [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
G-EQUAS 48/2A	41.9	2.2	5.2
G-EQUAS 48/2B	327	1.0	2.4

Day-to-day precision

For the determination of day-to-day precision, the materials of the interlaboratory trial were processed and analysed on six different days. The day-to-day precision data are presented in Table 6.

Tab. 6 Day-to-day precision for the determination of total selenium in urine (n = 6)

Material	Nominal concentration [µg/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
G-EQUAS 48/2A	41.9	5.8	14.9
G-EQUAS 48/2B	327	5.3	13.4

11.2 Accuracy

The accuracy of the method was determined by measuring the G-EQUAS interlaboratory-trial materials 48/2 as well as the NIST reference materials SRM 2670a in triplicate. The relative recovery rates thus obtained are given in Table 7.

Tab. 7 Relative recovery rates for the determination of total selenium in urine (n = 3)

Material	Spiked concentration [µg/l]	Measured concentration (Mean ± SD) [µg/l]	Relative recovery rate r [%]	
			Mean	Range
G-EQUAS 48/2A	41.9	42.4 ± 3.4	101	92.4–108.1
G-EQUAS 48/2B	327	332 ± 25.1	102	93.2–108.2
NIST SRM 2670a Low	8	7.3 ± 0.5	91	83.8–95.0
NIST SRM 2670a High	230	198 ± 12.8	86	79.6–89.7

11.3 Matrix effects

In order to check for matrix effects, ten individual urines with creatinine concentrations in the range of 0.3–2.5 g/l were spiked with 20 µg Se per litre of urine and analysed. Each sample was processed and analysed both with and without spiking. The selenium concentrations measured in the unspiked samples were subtracted from the determined concentrations in the spiked samples. The mean relative recovery rate in the individual urine samples was found to be 98.7 ± 7.6%. The determination of selenium in urine is not influenced by the composition of the urine matrix.

11.4 Limits of detection and quantitation

The limits of detection and quantitation were ascertained according to DIN 32645 (DIN 2008). To this end, an equidistant 10-point calibration with a concentration range of 0.2–2.0 µg Se/l was prepared in pooled urine. These samples were processed in conjunction with an unspiked pooled urine as a reagent blank and then analysed. The limits of quantitation and detection were calculated, per DIN 32645, from the standard deviation of the calibration function. The limits of detection and quantitation thus obtained are given in Table 8.

Tab. 8 Limits of detection and quantitation for the determination of total selenium in urine

Analyte	Detection limit [µg/l]	Quantitation limit [µg/l]
Selenium	0.2	0.5

11.5 Sources of error

The hereby described determination of selenium by ICP-MS may be influenced by clusters which exhibit the same mass/charge ratio as the selenium isotope used for quantification. As such, while the use of the isotope ^{80}Se may seem like a viable solution due to its isotope abundance, it was not used for the quantification of selenium within the framework of this method due to its interferences with the $^{40}Ar^{40}Ar^{+}$ and $^{79}B^{1}H$ clusters which also exhibit a mass/charge ratio of 80. By applying hydrogen as a reaction gas, polyatomic interferences, such as by $^{40}Ar^{38}Ar$ clusters, are minimised when the isotope ^{78}Se is used.

A reagent blank value was not observed under the described conditions. If germanium is present in the samples to be analysed, an alternative internal standard, such as rhodium (^{103}Rh) must be used.

12 Discussion of the method

The method described herein is based on a method for the determination of total selenium in urine by ICP-MS (Jäger et al. 2013; Jäger 2014). It is characterised by its high precision and robustness, whereby ICP-MS, as a considerably sensitive, element-specific detection method, enables the demonstrably sensitive measurement of selenium. Moreover, the quantitation limit of 0.5 µg Se/l allows for the measurement of background levels in the urine of the non-occupationally exposed general population.

After sample processing, the ICP-MS system, in combination with an autosampler, analyses the samples automatically. The method is thereby suitable for application in routine laboratories. Decomposition using a microwave-assisted digestion system is, however, time-consuming, since the decomposition solutions require cooling time in addition to the actual 30-minute decomposition and since the decomposition vessels must be rinsed. Furthermore, the rack positions in the microwave-assisted digestion system used during method development are limited. The Multiwave 3000 has a rotor in which a maximum of eight decomposition vessels can be heated in parallel. For high sample throughput, it is therefore recommended to use microwave-assisted digestion systems with a larger number of rack positions.

Thermal digestion using a heating block (e.g. ANALAB® HotPlate, ANALAB, Paris, France or DigiPREP, SCP SCIENCE, Bernd Kraft GmbH, Duisburg, Germany), using 5 ml of urine, 1.5 ml of ultra-pure water, 1.0 ml of 65% nitric acid, 3.0 ml of 30% hydrogen peroxide, and 0.5 ml of the ISTD spiking solution, may serve as a viable alternative. With this approach, a larger number of samples can be processed in parallel; for example, 48 samples can be heated simultaneously on an ANALAB® HotPlate heating block. Furthermore, the 50-ml polypropylene tubes used as decomposition vessels in thermal digestion are single-use products, meaning that it is not necessary to rinse the decomposition vessels after use. By performing digestion in a heating block, the total duration of selenium determination is drastically reduced and may be preferable to the microwave-assisted digestion system described here, particularly for routine application.

The mineralisation step converts all selenium species present in the urine into inorganic selenium, according to Wang et al. (2001), into selenite. The complete mineralisation of the organic material eliminates matrix influences on the analytical results and enables the correct measurement of both organic and inorganic selenium compounds. This process is especially important with regard to dimethyl selenide and dimethyl diselenide, insofar as these selenium species are present in the urine samples to be analysed.

Due to their volatility, both of the aforementioned methylated selenium species are increasingly transferred into the plasma due to the nebulisation in the spray chamber and thereby exhibit considerably higher signals compared with non-volatile compounds such as selenite. This effect, which was first described by Juresa et al. (2006), could be confirmed by the developers of this method. As such, total selenium concentrations of 44 urine samples from the general population–without digestion but diluted in an acidic medium–were 35% higher than those processed by microwave-assisted digestion as described in this method (Figure 2).

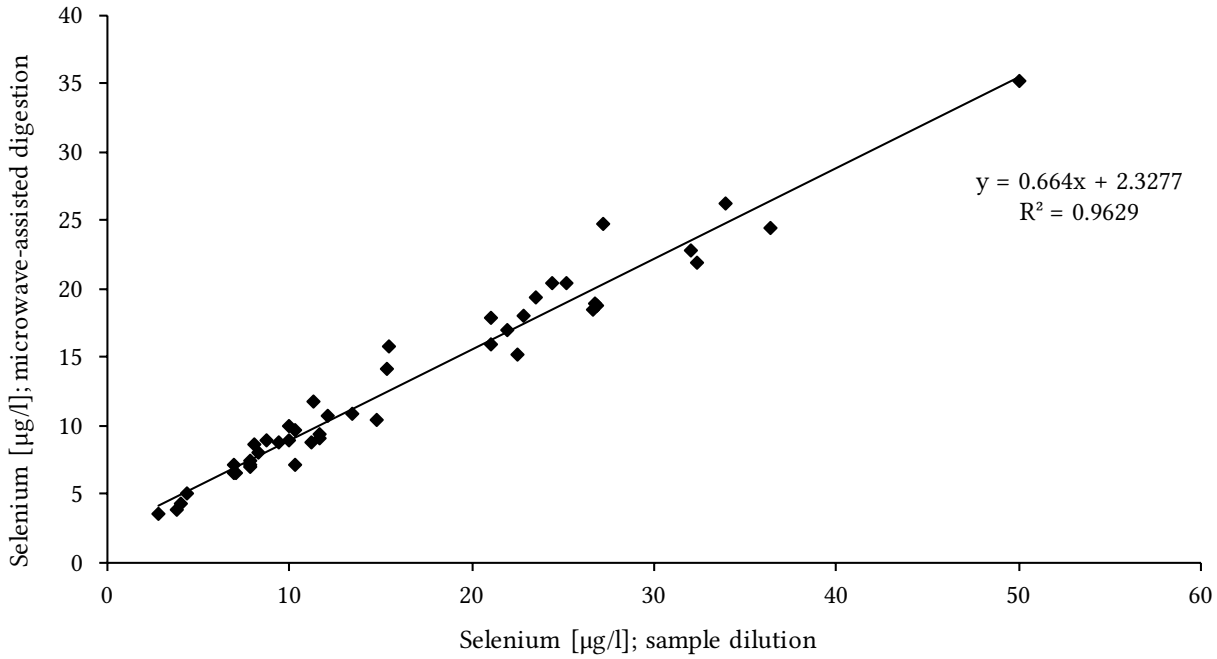

Fig. 2 Selenium concentrations following processing of urine samples with microwave-assisted digestion or only with dilution in an acidic medium (n = 44)

The method verifier also analysed urine samples from the general population (n = 38) both with microwave-assisted sample digestion and after dilution of the samples in an acidic medium. For this purpose, 500 µl of each sample was diluted 1 : 10 (v/v) with a solution containing 1% HNO_3, 1 µg rhodium/l as internal standard, and 1% ethanol (Heitland and Köster 2021). The addition of ethanol serves the purposes of matrix adjustment as well as the minimisation of carbon interferences. In contrast to the results of the method developers, comparable total selenium concentrations were obtained from both the microwave-digested and the diluted samples.

It is possible that the span of time from specimen collection to sample preparation as well as storage may influence the concentration of volatile selenium species; the varying results obtained by each laboratory may be attributable to these factors. Moreover, the type and proportion of organic selenium species in the urine samples depend on the absorbed selenium species as well as the route of uptake and individual metabolism.

In addition to using hydrogen as a reaction gas (ICP-MS; ^{78}Se), the method verifier also tested using oxygen as a reaction gas (ICP-MS/MS; $^{78}Se \longrightarrow {}^{94}SeO$) and obtained comparable selenium concentrations in the urine samples (n = 26) (Figure 3). The consistent results show that both hydrogen as reaction gas in ICP-MS and oxygen as reaction gas in ICP-MS/MS can be used to efficiently eliminate spectral interferences in the determination of selenium.

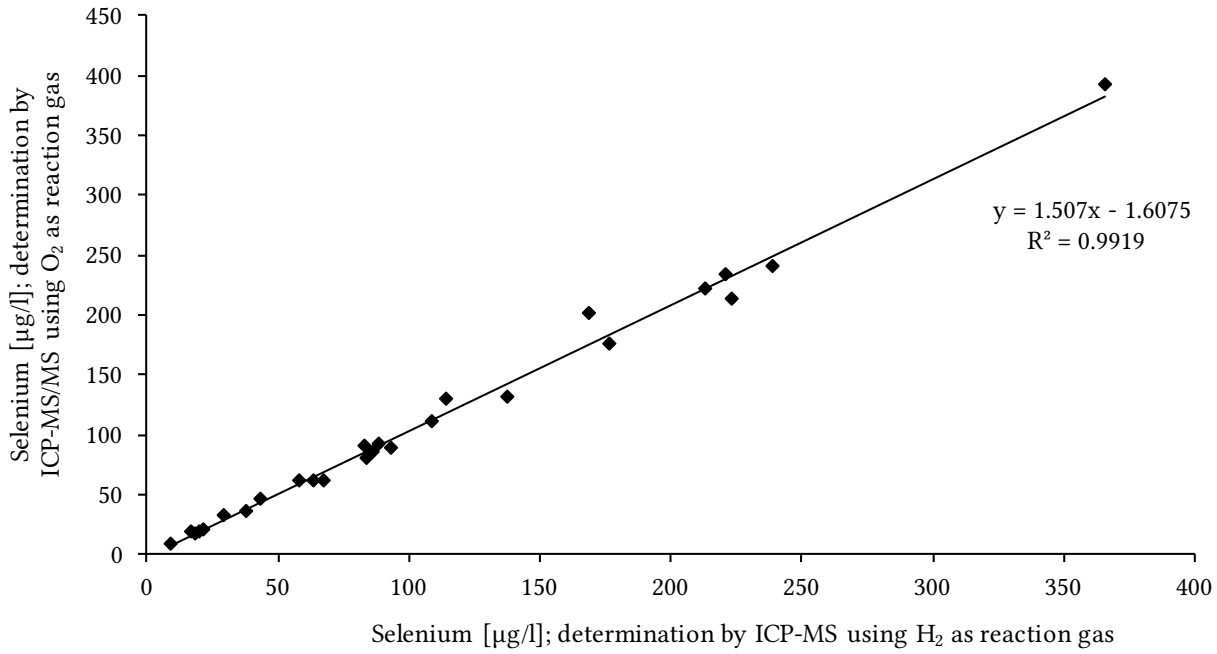

Fig. 3 Selenium concentrations after processing urine samples with microwave-assisted pressure digestion and using either hydrogen (ICP-MS; ^{78}Se) or oxygen (ICP-MS/MS; $^{78}Se \rightarrow {}^{94}SeO$) as the reaction gas (n = 26)

The dilution of the urine samples may also be a possibility when, in routine analysis, sample volumes of less than 5 ml are standard. In this case, the samples can be diluted in 1% ethanol and analysed as described above for matrix adjustment as well as to minimise interferences. During external method verification, it could be shown that selenium concentrations in urine can be correctly determined even after the dilution of small volumes of urine (e.g. 500 µl).

Participation in the "German External Quality Assessment Scheme" (G-EQUAS; https://app.g-equas.de/web/), an interlaboratory-comparison program offered by the German Society of Occupational and Environmental Medicine (*Deutsche Gesellschaft für Arbeits- und Umweltmedizin*, DGAUM) may serve as a measure for external quality assurance.

Instruments used Mass spectrometer with inductively coupled plasma and octopole reaction cell (Agilent 7500cx, Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany); ICP-MS autosampler (Agilent ASX-500 Series ICP-MS autosampler, Agilent Technologies Germany & Co. KG, Waldbronn, Germany); Microwave-assisted digestion system (Multiwave 3000 with 8SXF100 rotor, Anton Paar Germany GmbH, Ostfildern-Scharnhausen, Germany)

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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