The MAK-Collection for Occupational Health and Safety
DFG
GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Selenium and its compounds – Determination of selenosugar 1, selenosugar 2, selenomethionine, selenoethionine, methylselenic acid, *Se*-methylselenocysteine, and *Se*-methylselenoglutathione in urine by reversed-phase chromatography-ICP-MS

Biomonitoring Method – Translation of the German version from 2022

J. Hildebrand[1]
T. Jäger[1]
T. Göen[1,3,*]
B. Michalke[2]
A. Hartwig[4,*]
MAK Commission[5,*]

1 *Method development, Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute and Outpatient Clinic of Occupational, Social, and Environmental Medicine, Henkestraße 9–11, 91054 Erlangen, Germany*

2 *External verification, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Research Unit Analytical BioGeoChemistry, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany*

3 *Head of 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, Deutsche Forschungsgemeinschaft, Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute and Outpatient Clinic of Occupational, Social, and Environmental Medicine, Henkestraße 9–11, 91054 Erlangen, Germany*

4 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

5 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: T. Göen (thomas.goeen@fau.de), A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

selenium species; speciation analysis; biomonitoring; urine; HPLC; ICP-MS

Citation Note:
Hildebrand J, Jäger T, Göen T, Michalke B, Hartwig A, MAK Commission. Selenium and its compounds – Determination of selenosugar 1, selenosugar 2, selenomethionine, selenoethionine, methylselenic acid, *Se*-methylselenocysteine, and *Se*-methylselenoglutathione in urine by reversed-phase chromatography-ICP-MS. Biomonitoring Method – Translation of the German version from 2022. MAK Collect Occup Health Saf. 2022 Jun;7(2):Doc041. https://doi.org/10.34865/bi778249be7_2or

Manuscript completed:
11 Mar 2021

Publication date:
29 Jun 2022

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

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. This analytical method is used to determine relevant weakly polar or ionic organic selenium species in urine. They are separated by reversed-phase chromatography and subsequently detected using inductively coupled plasma-mass spectrometry (ICP-MS). Calibration is performed using standard solutions prepared in pooled urine, which are processed and analysed analogously to the samples. The analytical method allows for the selective and sensitive determination of seven selenium species without interferences by other species. The limits of quantitation for the determination of the individual species lie between 0.18 µg/l and 0.34 µg/l urine (as selenium) and are thereby sufficient to measure exposure levels which exceed the background exposure of the general population.

1 Characteristics of the method

Matrix	Urine
Analytical principle	High-performance liquid chromatography with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS)

Parameter and corresponding hazardous substances

Hazardous substances	CAS No.	Parameter	CAS No.
Selenium and its compounds	7782-49-2	Methyl-2-acetamido-2-deoxy-1-seleno-*β*-*D*-galactopyranoside (selenosugar 1, SeSug 1)	526222-32-2
		Methyl-2-acetamido-2-deoxy-1-seleno-*β*-*D*-glucopyranoside (selenosugar 2, SeSug 2)	408345-09-5
		Selenomethionine (SeMet)	1464-42-2
		Selenoethionine (SeEt)	6810-64-6
		Methylselenic acid (MeSeA)	28274-57-9
		Se-methylselenocysteine (MeSeC)	26046-90-2
		Se-methylselenoglutathione (MeSeG)	1207380-89-9

Reliability data

Selenosugar 1 (SeSug 1)

Within-day precision: Standard deviation (rel.) $s_w = 9.1\%$ or 5.8%
Prognostic range $u = 23.4\%$ or 14.9%
at a concentration of 1.3 µg or 4.9 µg per litre of urine (as selenium) and n = 6 determinations

Day-to-day precision [a)]: Standard deviation (rel.) $s_w = 5.4\%$
Prognostic range $u = 11.8\%$
at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations

Accuracy: Recovery rate (rel.) $r = 95.4\%$
at a spiked concentration of 1.2 µg per litre of urine (as selenium) and n = 11 determinations

Detection limit: 0.10 µg per litre of urine (as selenium)

Quantitation limit: 0.30 µg per litre of urine (as selenium)

[a)] Day-to-day precision data were collected by the external verifier of the method.

Selenosugar 2 (SeSug 2)

Within-day precision: Standard deviation (rel.) $s_w = 10.6\%$ or 5.2%
Prognostic range $u = 27.2\%$ or 13.4%
at a concentration of 1.2 µg or 4.3 µg per litre of urine (as selenium) and n = 6 determinations

Day-to-day precision [a]:	Standard deviation (rel.)	s_w = 1.7%
	Prognostic range	u = 4.4%
	at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 98.9%
	at a spiked concentration of 1.1 µg per litre of urine (as selenium) and n = 11 determinations	
Detection limit:	0.12 µg per litre of urine (as selenium)	
Quantitation limit:	0.34 µg per litre of urine (as selenium)	

[a] Day-to-day precision data were collected by the external verifier of the method.

Selenomethionine (SeMet)

Within-day precision:	Standard deviation (rel.)	s_w = 10.0% or 4.7%
	Prognostic range	u = 25.7% or 12.1%
	at a concentration of 0.8 µg or 3.7 µg per litre of urine (as selenium) and n = 6 determinations	
Day-to-day precision [a]:	Standard deviation (rel.)	s_w = 5.5%
	Prognostic range	u = 14.1%
	at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 97.4%
	at a spiked concentration of 0.8 µg per litre of urine (as selenium) and n = 11 determinations	
Detection limit:	0.06 µg per litre of urine (as selenium)	
Quantitation limit:	0.18 µg per litre of urine (as selenium)	

[a] Day-to-day precision data were collected by the external verifier of the method.

Selenoethionine (SeEt)

Within-day precision:	Standard deviation (rel.)	s_w = 9.1% or 3.6%
	Prognostic range	u = 23.4% or 9.26%
	at a concentration of 0.9 µg or 4.3 µg per litre of urine (as selenium) and n = 6 determinations	
Day-to-day precision [a]:	Standard deviation (rel.)	s_w = 4.9%
	Prognostic range	u = 12.6%
	at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 97.7%
	at a spiked concentration of 0.9 µg per litre of urine (as selenium) and n = 11 determinations	
Detection limit:	0.08 µg per litre of urine (as selenium)	
Quantitation limit:	0.22 µg per litre of urine (as selenium)	

[a] Day-to-day precision data were collected by the external verifier of the method.

Methylselenic acid (MeSeA)

Within-day precision:	Standard deviation (rel.)	s_w = 9.9% or 6.1%
	Prognostic range	u = 25.5% or 15.7%
	at a concentration of 1.2 µg or 4.7 µg per litre of urine (as selenium) and n = 6 determinations	
Day-to-day precision [a]:	Standard deviation (rel.)	s_w = 4.4%
	Prognostic range	u = 11.3%
	at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 110%
	at a spiked concentration of 0.8 µg per litre of urine (as selenium) and n = 11 determinations	
Detection limit:	0.13 µg per litre of urine (as selenium)	
Quantitation limit:	0.33 µg per litre of urine (as selenium)	

[a] Day-to-day precision data were collected by the external verifier of the method.

Se-methylselenocysteine (MeSeC)

Within-day precision:	Standard deviation (rel.)	s_w = 7.6% or 4.1%
	Prognostic range	u = 19.5% or 10.5%
	at a concentration of 1.0 µg or 4.1 µg per litre of urine (as selenium) and n = 6 determinations	
Day-to-day precision [a]:	Standard deviation (rel.)	s_w = 4.6%
	Prognostic range	u = 11.8%
	at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy:	Recovery rate (rel.)	r = 107%
	at a spiked concentration of 0.9 µg per litre of urine (as selenium) and n = 11 determinations	
Detection limit:	0.08 µg per litre of urine (as selenium)	
Quantitation limit:	0.22 µg per litre of urine (as selenium)	

[a] Day-to-day precision data were collected by the external verifier of the method.

Se-methylselenoglutathione (MeSeG)

Within-day precision:	Standard deviation (rel.)	s_w = 6.6% or 7.0%
	Prognostic range	u = 17.0% or 18.0%
	at a concentration of 1.1 µg or 5.3 µg per litre of urine (as selenium) and n = 6 determinations	
Day-to-day precision [a]:	Standard deviation (rel.)	s_w = 7.4%
	Prognostic range	u = 19.0%
	at a concentration of 5 µg per litre of urine (as selenium) and n = 6 determinations	

Accuracy:	Recovery rate (rel.) $r = 86.0\%$ at a spiked concentration of 1.3 µg per litre of urine (as selenium) and n = 11 determinations
Detection limit:	0.07 µg per litre of urine (as selenium)
Quantitation limit:	0.21 µg per litre of urine (as selenium)

[a] Day-to-day precision data were collected by the external verifier of the method.

2 General information on selenium and its compounds

Selenium (Se; relative atomic mass: 78.97; atomic number: 34) is an element that is found ubiquitously in the environment and which occurs most frequently in compounds in the oxidation states −II, +IV, and +VI. The most important producers of selenium are the USA, Canada, and Japan, which altogether generate about 70% of world production. About 45% of selenium is then used in electrical engineering, 20% in the production of pigments, 8% in the chemical industry, and about 27% in the ceramics and glass industries as well as for other industrial purposes (Butterman and Brown 2004; RÖMPP-Redaktion and Hartwig 2006). Each year, 1000 to 10 000 tonnes of selenium are either produced in or imported into the European Economic Area (ECHA 2022).

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 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 absorption rates for inorganic selenites and selenates lie between 62–84% and 92–94%, respectively, and those for organic selenium compounds lie between 75–95% (Rettenmeier 2019).

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 usually carried out by examining selenium levels in blood plasma. There are only a few studies on renal selenium excretion following occupational exposure (Göen and Greiner 2018), most of which consist of determining total selenium content in employee urine (Table 1).

Tab. 1 Selenium concentrations in urine following occupational exposure

Collective (Sample number n)	Sample matrix	Selenium (mean ± SD (range))		References
		Workers	Controls	
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

Tab. 1 *(continued)*

Collective (Sample number n)	Sample matrix	Selenium (mean ± SD (range))		References
		Workers	Controls	
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)]		
Selenium-rectifier manufacture, England (1517 samples from 200–300 workers; 793 controls)	Urine	84 µg/l	34 ± 24 µg/l (0–150 µg/l)	Glover 1967
Copper refinery, Canada (20)	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

[a)] Median (range)

For selenium and its inorganic compounds, the Commission has derived a biological tolerance value (BAT value) of 150 µg selenium/l plasma and a maximum workplace concentration (MAK value) of 0.02 mg selenium/m^3 E (as selenium). In addition, selenium and its inorganic compounds have been classified as Category 3 carcinogens and are designated with an "H," as they are readily absorbed through the skin. Details on the toxicological evaluation can be found in the corresponding MAK documentation published by the Commission (DFG 2021; Hartwig 2014, 2015). Furthermore, the Commission has derived a biological reference value (BAR) 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).

The absorbed inorganic and organic selenium compounds are reduced to selenide, whereby this reaction is glutathione-dependent for selenites and selenates. Selenide is the central metabolic selenium species (see Figure 1) and serves as a common source for the synthesis of selenoproteins and selenosugars (Birringer et al. 2002; Fairweather-Tait et al. 2011; Navarro-Alarcon and Cabrera-Vique 2008; Ohta and Suzuki 2008). Excess selenide can be eliminated via three metabolic pathways. The most important elimination products of selenium are the selenium-containing sugars (Francesconi and Pannier 2004; Kuehnelt et al. 2005). So far, the three compounds methyl-2-acetamido-2-deoxy-1-seleno-*β*-*D*-galactopyranoside (SeSug 1), methyl-2-acetamido-2-deoxy-1-seleno-*β*-*D*-glucopyranoside (SeSug 2), and methyl-2-amino-2-deoxy-1-seleno-*β*-*D*-galactopyranoside (SeSug 3) have been detected in human urine, with SeSug 1 being the most prominent selenosugar (Hildebrand et al. 2020; Jäger et al. 2013). Another metabolic pathway involves methyltransferases, which convert the intermediate selenide to methylated compounds such as monomethylselenol, dimethylselenide, and the trimethylselenonium ion (TMSe) (Kremer et al. 2005; Ohta and Suzuki 2008). Dimethylselenide is excreted via the skin (Ganther 1986) and lungs (Jiang et al. 1983), and TMSe is excreted renally (Kuehnelt et al. 2006). The excretion of TMSe via the kidneys is subject to major interindividual variations ranging from trace concentrations up to the primary elimination product (Gammelgaard and Jøns 2000; Kuehnelt et al. 2006; Lu and Holmgren 2009). In a study in Germany, TMSe was either not or only marginally detectable in 80% of the study participants, but was found to be the main metabolite in the remaining 20% (Jäger et al. 2013). For the selenium species selenate, it has been shown that a large proportion of orally ingested selenate is excreted renally in its unmetabolised form, and is thus not available to the central selenide pool (Gammelgaard et al. 2012; Jäger et al. 2016). A portion of selenium is also excreted via the biliary tract and is subject to enterohepatic circulation (RKI 2006).

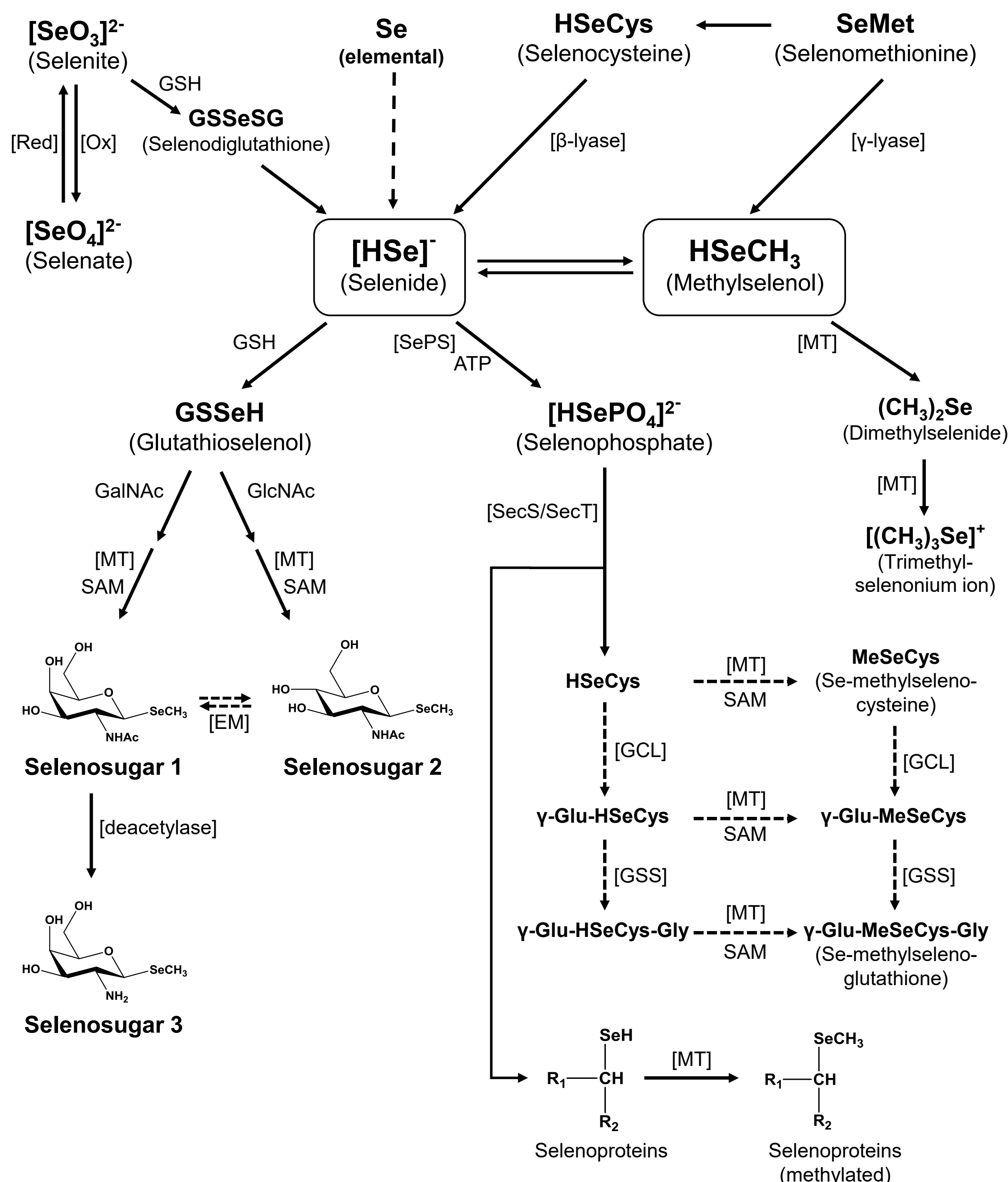

Fig. 1 Metabolism scheme of selenium according to Navarro-Alarcon and Cabrera-Vique (2008) as well as Fairweather-Tait et al. (2011). EM: epimerase; GalNAc: *N*-acetylgalactosamine; GlcNAc: *N*-acetylglucosamine; GCL: glutamate-cysteine ligase; GSH: glutathione; GSS: glutathione synthetase; MT: methyltransferase; SAM: *S*-adenosyl methionine; SecS/SecT: *L*-seryl-tRNASec selenium transferase; SePS: selenophosphate synthetase

The individual selenium compounds (species), which are absorbed by the body or formed metabolically, may differ considerably in terms of their physical properties as well as their toxicological potential (Nuttall 2006). The determining factor for bioavailability and for biochemical or toxic effects is the type and amount of the selenium species, which is not reflected by the total concentration of selenium in urine (Cornelis et al. 1993; Kiss and Odani 2007; Lund 1990; Michalke 2002 a, b). Therefore, an analytical differentiation of the relevant selenium species found in urine is imperative for the evaluation of both occupational and non-occupational exposure.

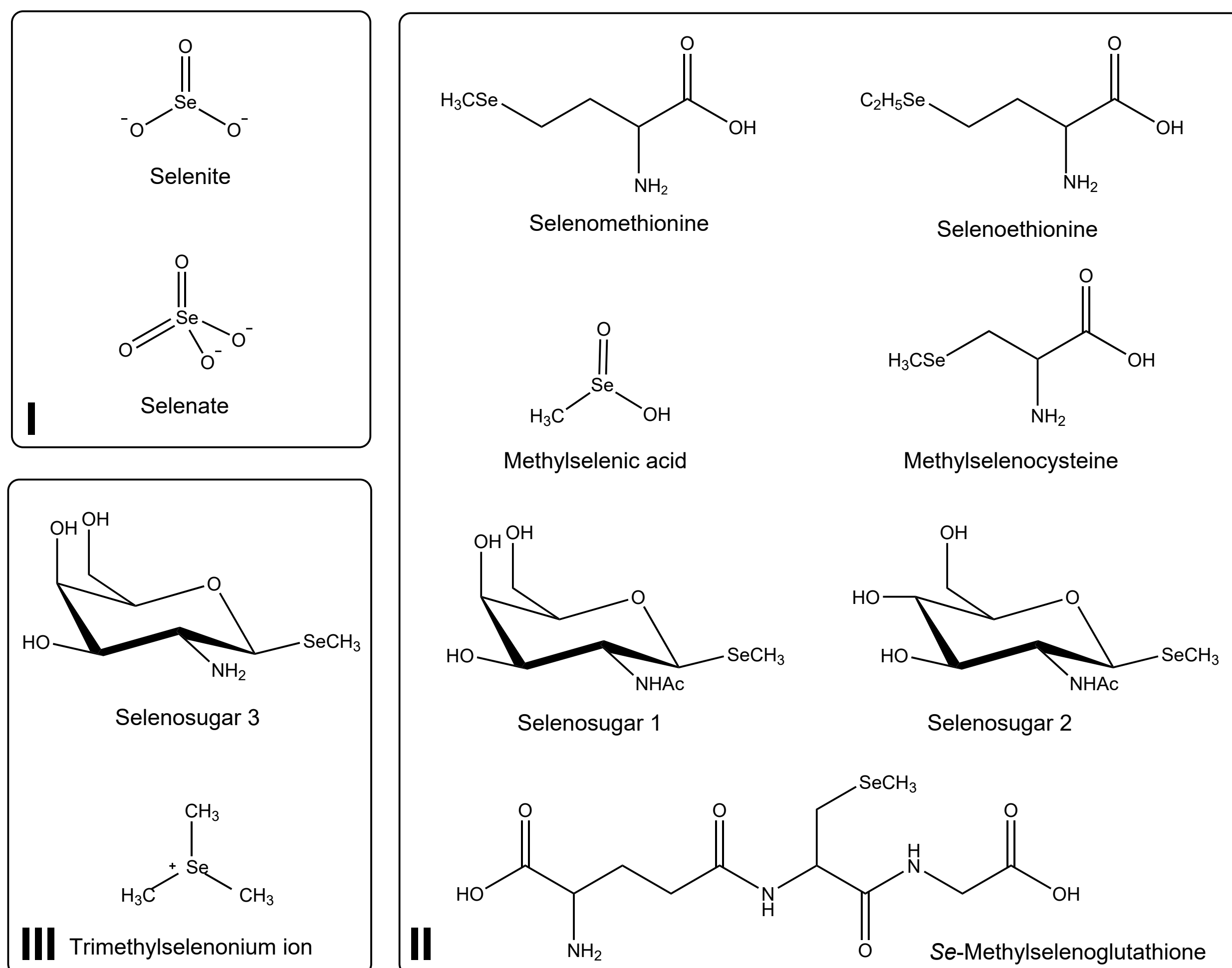

Fig. 2 Structures of the selenium species which can be determined with the three submethods

The "Analyses in Biological Materials" working group developed and validated three methods allowing for the quantification of a total of eleven selenium species which utilise various liquid-chromatographic separation mechanisms (I: anion-exchange chromatography; II: reversed-phase chromatography; III: cation-exchange chromatography). Due to these different liquid-chromatographic separation mechanisms, the three submethods are published separately. Figure 2 shows the structures of the selenium species which can be determined with the three submethods.

The determination of SeSug 1, SeSug 2, SeMet, SeEt, MeSeA, MeSeC, and MeSeG is performed using reversed-phase chromatography and is described as part of the hereby presented Submethod II.

SeSug 1 is the selenium species which is present in the highest concentration in the urine of the general population. As such, SeSug 1 could be detected in all urine samples from the German general population analysed by Jäger et al. (2013) (median: 1.44 µg Se/l; range: 0.13–5.05 µg Se/l; n = 47). These concentrations correspond with values published by Letsiou et al. (2007) and Lu et al. (2012) of 1.3 µg Se/l (n = 1) and < 0.6–1.3 µg Se/l (n = 2), respectively. The background concentrations in Austria, as published by Lajin et al. (2016), slightly exceeded these values (median: 3.7 µg Se/l; range: 0.9–24.4 µg Se/l; n = 8). Following oral supplementation, the excretion of SeSug 1 increases considerably: 200 µg of selenium (nutritional supplement, not specified) yields an excretion of 62 µg Se/l (n = 1) (Letsiou et al. 2007); 200 µg Se as selenium yeast yields excretions in the range of 11.0–31.2 µg Se/l (n = 2) (Lu et al. 2012); 200 µg or 400 µg selenium as selenite yields average concentrations of 22.5 or 80.8 µg Se/l, respectively (n = 8) (Lajin et al. 2016). When even higher

amounts of selenium are given (1000 µg of selenium as selenite), urinary SeSug 1 concentrations increase even further (median: 218 µg Se/l; n = 1) (Kuehnelt et al. 2005).

In a supplementation study with one test subject, the urinary SeSug 2 concentration was found to be below the detection limit at the start of the study and, after an oral dosage of 1000 µg of selenium (as sodium selenite, *L*-SeMet, *DL*-SeMet), increased to between 1.9 and 3.8 µg Se/l (Kuehnelt et al. 2005).

MeSeA is not excreted with the urine as a product of metabolism, but rather arises during the oxidative degradation of SeSug 1 in urine (Hildebrand et al. 2020; Ogra et al. 2003). To date, no data have yet been published on MeSeA concentrations in native urine samples.

The background concentrations of SeMet have been investigated in several studies, though urinary concentrations were always found to be below the detection limit (Gómez-Ariza et al. 2000; Jäger et al. 2013; Lu et al. 2012; Mao et al. 2012; Zheng et al. 2002). Following selenium supplementation, however, SeMet could be detected in urine in the lower µg/l range. In this case, SeMet was detected in urine after supplementation with 100 µg selenium as selenate or with 100 µg selenium as selenium yeast, whereby the concentrations lied between the detection limit and the quantitation limit (Eichler et al. 2015). SeMet was detected at a concentration of about 11 µg Se/l following a dosage of 400 µg *DL*-SeMet (Cao et al. 2001) and at a concentration of 21 µg Se/l following a dosage of 1000 µg *DL*-SeMet (Kuehnelt et al. 2005).

SeEt could not be detected in urine samples from the general population (Jäger et al. 2013) or could only be detected in low concentrations, specifically between the detection and quantitation limits (Zheng et al. 2002).

MeSeC could not be detected in the urine of the non-occupationally exposed general population nor in those who do not ingest selenium via nutritional supplements (Mao et al. 2012); in other cases, it could only be detected in very low concentrations. A study carried out in Germany quantified the analyte in 9 of 47 urine samples, whereby the median lied at 0.13 µg Se/l (range: 0.03–0.41 µg Se/l) (Jäger et al. 2013). Urinary MeSeC concentration increases after supplementation, such that an oral dosage of 100 µg of selenium as selenium yeast yielded an average concentration of 3.68 µg Se/l (n = 5) (Eichler et al. 2015) or that an oral dosage of 200 µg selenium as MeSeC yielded values up to 6.8 µg Se/l (Kokarnig et al. 2015).

In *in vitro* experiments, MeSeG was detected by Marschall et al. (2017) as a metabolite of MeSeC. In a study performed in Germany, however, it could not be detected in urine samples from the general population (Jäger et al. 2013).

3 General principles

The analytical method described herein is used to determine relevant weakly polar or ionic organic selenium species in urine. Selenosugar 1, selenosugar 2, selenomethionine, selenoethionine, methylselenic acid, *Se*-methylselenocysteine, and *Se*-methylselenoglutathione are separated by reversed-phase chromatography and subsequently quantified using inductively coupled plasma-mass spectrometry (ICP-MS). Calibration is performed using standard solutions, which are prepared in pooled urine, processed, and analysed analogously to the samples.

4 Equipment, chemicals, and solutions

4.1 Equipment

- HPLC system (e.g. Agilent 1200 series with a binary pump (G1312A) and an autosampler (G1329A), Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany)
- Inductively coupled plasma-mass spectrometer with collision/reaction cell (e.g. Agilent 7500cx, Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany)

- Analytical column: Phenomenex Luna C18(2) (150 × 4.6 mm; 3 µm) with Phenomenex Security Guard system (C18; 4 × 3.0 mm) (e.g. Phenomenex Ltd. Deutschland, Aschaffenburg, Germany)
- Ultrasonic bath (e.g. VWR International GmbH, Darmstadt, Germany)
- Vortex mixer (e.g. Heidolph Instruments GmbH & Co. KG, Schwabach, Germany)
- Precision balance (e.g. Mettler-Toledo GmbH, Gießen, Germany)
- Cellulose filter, pore size of 45 µm, membrane of mixed cellulose esters (e.g. Merck KGaA, Darmstadt, Germany)
- 15- and 50-ml polypropylene tubes (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)
- 0.7-ml snap-top polypropylene microvials (e.g. VWR International GmbH, Darmstadt, Germany)
- 1.5-ml vials (e.g. Eppendorf AG, Hamburg, Germany)
- 10-, 100-, and 1000-ml volumetric flasks (e.g. VWR International GmbH, Darmstadt, Germany)
- Urine cups (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)
- Various pipettes and Multipettes® with matching pipette tips (e.g. Eppendorf AG, Hamburg, Germany)

4.2 Chemicals

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

- Malonic acid for synthesis (e.g. No. 800387, Merck KGaA, Darmstadt, Germany)
- Methanol Supelco LiChrosolv® (e.g. No. 106035, Merck KGaA, Darmstadt, Germany)
- Ultra-pure water (e.g. Milli-Q® Direct Water Purification System, Merck KGaA, Darmstadt, Germany)
- Pooled urine from individuals neither occupationally exposed to selenium nor who ingest selenium via nutritional supplements; refrigerated for 48 h
- Argon 5.0 (Linde GmbH, Pullach, Germany)
- Hydrogen 5.0 (Linde GmbH, Pullach, Germany)

4.3 Reference materials

- Methyl-2-acetamido-2-deoxy-1-seleno-*β*-*D*-galactopyranoside > 95% (e.g. custom synthesis, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany)
- Methyl-2-acetamido-2-deoxy-1-seleno-*β*-*D*-glucopyranoside > 95% (e.g. custom synthesis, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany)
- *Se*-methylselenoglutathione > 95% (e.g. custom synthesis, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany)
- *Se*-methylselenocysteine hydrochloride (2-amino-3-(methylselenyl)propionic acid hydrochloride) ≥ 95% (e.g. No. M6680, Merck KGaA, Darmstadt, Germany)
- Seleno-*D*,*L*-methionine (2-amino-4-(methylselenyl)butyric acid) ≥ 99% (e.g. No. S3875, Merck KGaA, Darmstadt, Germany)
- Seleno-*D*,*L*-ethionine (2-amino-4-(ethylselenyl)butyric acid) ≥ 95% (e.g. No. E5139, Merck KGaA, Darmstadt, Germany)
- Methylselenic acid 95% (e.g. No. 541281, Merck KGaA, Darmstadt, Germany)
- Selenium ICP standard Certipur®, SeO_2 in 2–3% HNO_3, 1000 mg Se/l (e.g. No. 1.70350, Merck KGaA, Darmstadt, Germany)

4.4 Solutions

- Malonic acid solution (1 mol/l)
  10.4 g of malonic acid are weighed into a 100-ml volumetric flask and dissolved in ultra-pure water. The flask is made up to the mark with ultra-pure water.

- Malonic acid solution (4 mmol/l) with 0.1% methanol
  Approximately 900 ml of ultra-pure water are placed in a 1000-ml volumetric flask, and 4 ml of the malonic acid solution as well as 1 ml of methanol are added. The flask is then made up to the mark with ultra-pure water. The eluent is freshly prepared for each analysis, filtered prior to use, and degassed in an ultrasonic bath for five minutes.

4.5 Calibration standards

- Stock solutions (1000 mg Se/l)
  The indicated amounts of each reference substance, as given in Table 2, are each weighed into a 10-ml volumetric flask. The flasks are made up to the mark with ultra-pure water.
  With regard to selenium, the solutions thus prepared contain about 1 g Se/l. In order to determine the exact selenium concentration, the stock solutions are measured against an ICP single-element standard to correct for any potential weighing errors.

Tab. 2 Molar masses and weigh-ins of the reference substances for the preparation of the corresponding stock solutions for the determination of SeSug 1, SeSug 2, SeMet, SeEt, MeSeA, MeSeC, and MeSeG in urine

Reference substance	Molar mass [g/mol]	Weigh-in [mg]
SeSug 1	299.2	38
SeSug 2	299.2	38
SeMet	196.1	25
SeEt	209.1	26
MeSeA	127.0	16
MeSeC	214.1	27
MeSeG	369.3	47

- Working solutions (10 mg Se/l)
  Depending on the measured concentrations of the stock solutions, about 1000 µl of each stock solution are pipetted into a 100-ml volumetric flask. The flasks are then made up to the mark with ultra-pure water.

- Spiking solution I (1 mg Se/l)
  100 µl of each working solution are pipetted into a 1.5-ml vial. Subsequently, 300 µl of ultra-pure water are added and the solution is thoroughly mixed.

- Spiking solution II (0.1 mg Se/l)
  100 µl of spiking solution I are placed in a 1.5-ml vial with 900 µl of ultra-pure water and mixed.

The stock and working solutions of the analytes are stored at −18 °C. Regarding SeSug 1 and SeSug 2, the method user must ensure the stability of the analytes in the prepared solutions in cases of long-term storage. The concentration of MeSeA may increase as a result of the degradation of SeSug 1 and SeSug 2.

In order to prepare the calibration standards, spiking solutions I and II are brought to a final volume of 10 ml using pooled urine according to the pipetting scheme given in Table 3. The spiking and calibration solutions are freshly

prepared for each analytical run. The calibration standards are processed analogously to the samples as described in Section 5.2.

Tab. 3 Pipetting scheme for the preparation of calibration solutions for the determination of SeSug 1, SeSug 2, SeMet, SeEt, MeSeA, MeSeC, and MeSeG in urine

Calibration standard	Spiking solution I [µl]	Spiking solution II [µl]	Pooled urine [ml]	Concentration [µg Se/l]
0	–	–		0
1	–	25		0.25
2	–	50		0.5
3	–	200	ad 10	2
4	50	–		5
5	100	–		10
6	250	–		25

5 Specimen collection and sample preparation

5.1 Specimen collection

The urine samples are collected in urine cups. Due to the instability of SeSug 1 and SeSug 2, the samples should be – if possible – processed and analysed directly following specimen collection. If this is not possible, the urine samples should be stored in the dark and frozen following specimen collection, and are to be analysed as soon as possible. In such cases, the method user must take measures to ensure the stability of SeSug 1 and SeSug 2 in the samples (see Section 11.4).

5.2 Sample preparation

The urine samples are, if necessary, brought to room temperature and thoroughly mixed. 300 µl of the sample are transferred into a 0.7-ml polypropylene microvial and mixed with an equal amount of eluent. The sample is thoroughly mixed for ten seconds on a vortex mixer. Of the sample thus diluted, 50 µl are applied for analysis.

6 Operational parameters

Analytical determination was performed on an HPLC system with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS).

6.1 Liquid chromatography

Analytical column:	Phenomenex Luna C18(2) (150 × 4.6 mm; 3 µm) with Phenomenex Security Guard system (C18; 4 × 3.0 mm)
Separation principle:	Reversed-phase chromatography
Eluent:	Malonic acid solution (4 mmol/l) with 0.1% methanol
Flow rate:	0–8 min: 0.6 ml/min 8–16 min: 0.9 ml/min
Injection volume:	50 µl

Runtime: 16 min

The given parameters must be optimised according to the manufacturer's specifications.

6.2 Inductively coupled plasma-mass spectrometry

Rf power:	1500 W
Nebuliser:	MikroMist
Spray chamber:	Scott Quartz
Spray-chamber temperature:	10 ℃
Carrier gas:	0.9 l argon/min
Make-up gas:	0.2 l argon/min
Sampling/skimmer cone:	Nickel
Reaction/collision gas:	H_2
Reaction/collision-gas flow rate:	1.5 ml/min
Analysis mode:	Time-resolved analysis
Number of measurements per mass trace (repetition):	1
Parameter-specific settings:	see Table 4

The instrument-specific parameters must be determined and adjusted by the method user for the specific ICP-MS system utilised. The parameters given in this section have been determined and optimised for the device configuration employed during method development. The measurements for this method are performed in collision-cell mode with hydrogen as the collision gas. As a rule, the ICP-MS should undergo the daily optimisation routine according to the manufacturer's specifications and achieve the given specification values which vary with each device manufacturer. A dwell time of 100–150 ms should be selected for sufficient chromatographic resolution. In principle, other nebulisers may be used for sample introduction. The selected mass traces and retention times are summarised in Table 4. The mass trace ^{78}Se is used for quantification.

Tab. 4 Retention times and und parameter-specific settings for the determination of MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG in urine

Analyte	Retention time [min]	Analysed mass trace	Measurement time per mass trace [s]
MeSeC	3.4	^{78}Se	0.5
		^{82}Se	0.3
SeMet	4.8	^{78}Se	0.5
		^{82}Se	0.3
MeSeA	5.8	^{78}Se	0.5
		^{82}Se	0.3
SeSug 2	8.8	^{78}Se	0.5
		^{82}Se	0.3
SeEt	9.8	^{78}Se	0.5
		^{82}Se	0.3
SeSug 1	10.5	^{78}Se	0.5
		^{82}Se	0.3

Tab. 4 *(continued)*

Analyte	Retention time [min]	Analysed mass trace	Measurement time per mass trace [s]
MeSeG	11.6	^{78}Se	0.5
		^{82}Se	0.3

7 Analytical determination

50 µl of each sample prepared as described in Section 5.2 are injected into the HPLC-ICP-MS system. Identification of the selenium species is based on their retention times. The retention times listed in Table 4 can only serve as a point of reference. Users must ensure proper separation performance of the HPLC column employed and the resulting retention behaviour of the analytes. A reagent blank (ultra-pure water instead of a urine sample) is included in each analytical run. Figure 3 shows a representative chromatogram of a urine sample spiked with the selenium species that can be determined with this method.

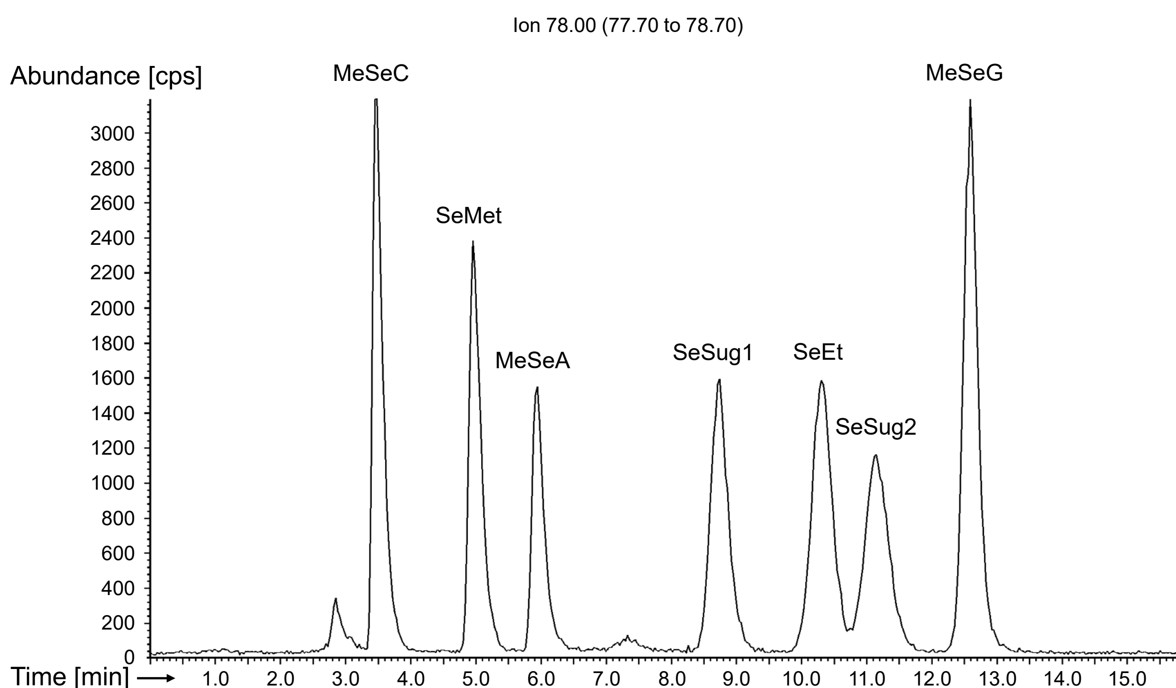

Fig. 3 Chromatogram of a urine sample spiked with MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG (each with 25 µg Se/l)

8 Calibration

The calibration standards (see Section 4.5) are processed analogously to the urine samples (see Section 5.2) and analysed. Calibration curves are obtained by plotting the peak areas of the analytes against the corresponding concentration of the calibration solution. The calibration curves of the selenium species are linear from 0.25 to 25 µg/l (as selenium) under the described analytical conditions. Figure 4 shows representative calibration curves for the selenium species in urine that can be determined with this method.

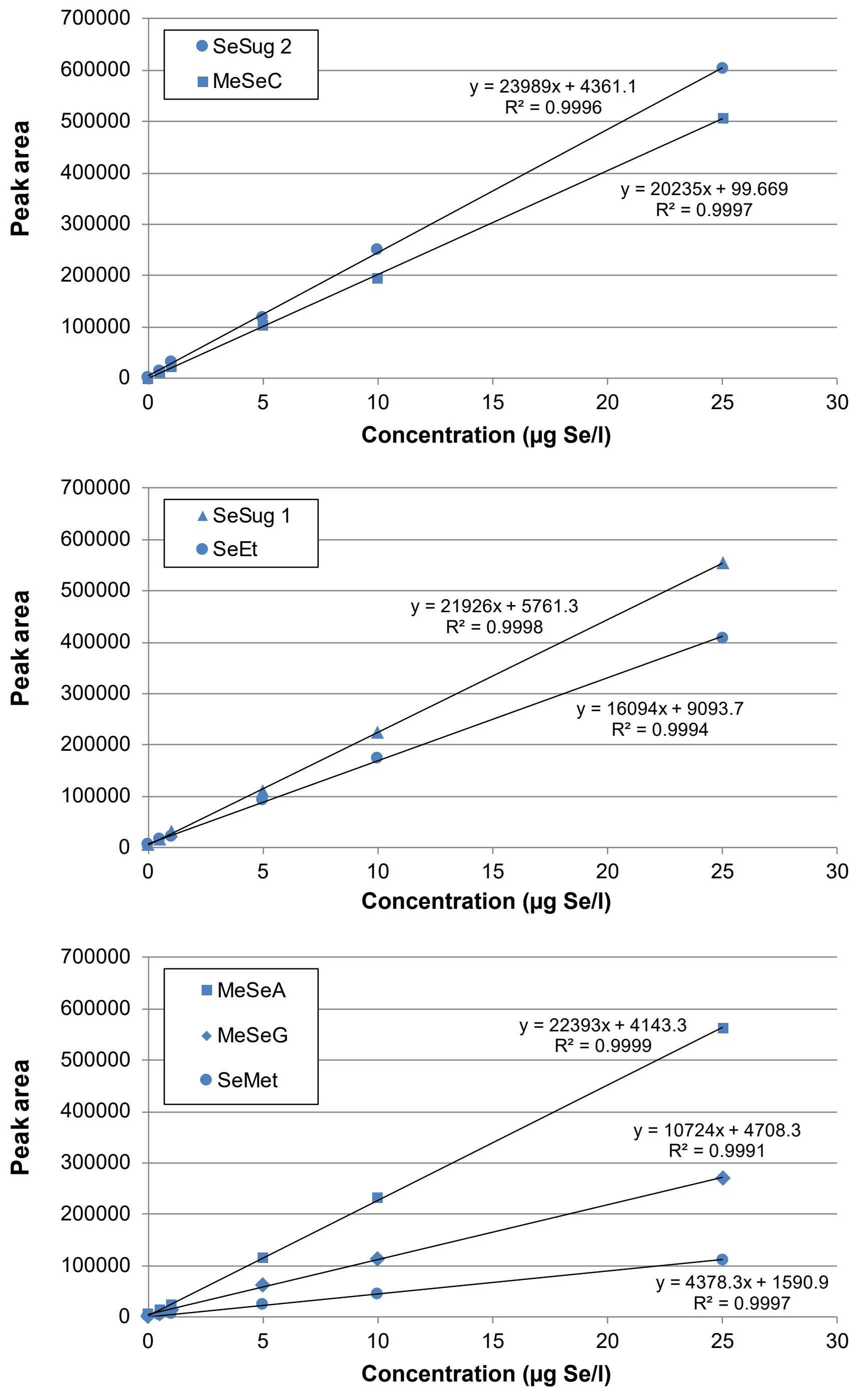

Fig. 4 Calibration curves for the determination of MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG in urine

9 Calculation of the analytical results

The analyte concentration of a sample in µg/l urine (as selenium) can be calculated by inserting the peak area of the corresponding analyte into the calibration function of the respective analytical run. It may be necessary to adjust the calibration range to the expected range of 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 control 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).

In order to assure the quality of the analytical results, two quality-control samples with different analyte concentrations are processed and analysed parallel to the samples as part of each analytical run. Since control material is not currently commercially available, the material must be prepared in the in-house laboratory using pooled urine from individuals who are neither occupationally exposed to selenium nor ingest selenium via nutritional supplements. The pooled urine is spiked with 1.0 µg/l (as selenium; Q_{low} – low quality-control material) or 4.0 µg/l (as selenium; Q_{high} – high quality-control material) of each species. The quality-control materials thus prepared are aliquoted to 300 µl and frozen at −18 ℃ until use.

The nominal values and tolerance ranges of the quality-control materials are determined within a pre-analytical period (one analysis of each quality-control material 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

Within-day precision

For the determination of within-day precision, the quality-control materials were processed and analysed six times in parallel on one day. The within-day precision data thus obtained are summarised in Table 5.

Tab. 5 Within-day precision for the determination of MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG in urine (n = 6)

Analyte	Spiked concentration [µg Se/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
MeSeC	1.0	7.6	19.5
	4.1	4.1	10.5
SeMet	0.8	10.0	25.7
	3.7	4.7	12.1
MeSeA	1.2	9.9	25.5
	4.7	6.1	15.7
SeSug 2	1.2	10.6	27.3
	4.3	5.2	13.4

Tab. 5 *(continued)*

Analyte	Spiked concentration [µg Se/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
SeEt	0.9	9.1	23.4
	4.3	3.6	9.26
SeSug 1	1.3	9.1	23.4
	4.9	5.8	14.9
MeSeG	1.1	6.6	17.0
	5.3	7.0	18.0

Day-to-day precision

Day-to-day precision data were collected by the external verifier of the method. Urine samples were spiked with 5 µg/l of each analyte and processed and analysed on six different days. The data thus obtained are presented in Table 6.

Tab. 6 Day-to-day precision for the determination of MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG in urine (n = 6)

Analyte	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
MeSeC	4.6	11.8
SeMet	5.5	14.1
MeSeA	4.4	11.3
SeSug 2	1.7	4.4
SeEt	4.9	12.6
SeSug 1	5.4	13.9
MeSeG	7.4	19.0

11.2 Accuracy

In order to ascertain the accuracy of the method, ten individual urines were spiked with the selenium species at a concentration of 2 µg/l (as selenium). The unspiked urines were additionally processed and analysed. Relative recovery was calculated using the determined concentrations of the spiked urines, subtracting any background analyte levels found in the unspiked urines. The relative recovery rates thus obtained are given in Table 7.

Tab. 7 Relative recovery rates for the determination of MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG in urine

Analyte	Mean rel. recovery r [%]	Range [%]
MeSeC	107	104–109
SeMet	97.4	78.4–116
MeSeA	110	94.6–126
SeSug 2	98.9	90.8–107
SeEt	97.7	92.0–103
SeSug 1	95.4	74.8–116
MeSeG	86.0	82.8–89.2

11.3 Limits of detection and quantitation

The limits of detection and quantitation were determined according to DIN 32645 (DIN 2008). To this end, an equidistant 10-point calibration was established, processed, and analysed in conjunction with a blank value (unspiked pooled urine). Table 8 shows the detection and quantitation limits obtained for the selenium species that can be determined using this method.

Tab. 8 Limits of detection and quantitation for the determination of MeSeC, SeMet, MeSeA, SeSug 2, SeEt, SeSug 1, and MeSeG in urine (n = 3)

Analyte	Detection limit [µg Se/l]	Quantitation limit [µg Se/l]
MeSeC	0.08	0.22
SeMet	0.06	0.18
MeSeA	0.13	0.33
SeSug 2	0.12	0.34
SeEt	0.08	0.22
SeSug 1	0.10	0.30
MeSeG	0.07	0.21

11.4 Sources of error

During the determination of day-to-day precision, the method developers found greater deviations in the measured values for the analytes SeSug 1, SeSug 2, and MeSeA compared to the external verifiers of the method. The worse precision data can likely be attributed to the degradation of SeSug 1 and SeSug 2 and the resultant formation of MeSeA as a degradation product. Microbial, chemical, and physical factors all play a role in the degradation of SeSug 1 and SeSug 2, which may take place during the long-term storage of urine samples (Hildebrand et al. 2020; Juresa et al. 2006). Both dimethyldiselenide (Juresa et al. 2006) and MeSeA (Hildebrand et al. 2020; Ogra et al. 2003) were identified as degradation products of SeSug 1 and SeSug 2. To prevent the decomposition of the selenosugars, the urine samples should be processed and analysed as quickly as possible following specimen collection.

If the storage of the urine samples cannot be avoided, the samples should be stored in the dark and refrigerated, if not frozen (Juresa et al. 2006). As a rule, SeSug 1 and SeSug 2 can be stabilised in the urine samples by adjusting their pH values and adding sodium azide (pH = 5.5; 0.1% sodium azide) (Hildebrand et al. 2020). The stabilisation of the selenosugars was successfully tested by Juresa et al. (2006) by storing the urines at −80 ℃ or the lyophilised urine samples at −20 ℃. The authors further recommended adding sodium azide or passing nitrogen through the samples.

Given the decomposition of SeSug 1 and SeSug 2, the quality-control materials can only be used over longer periods of time insofar as appropriate measures are taken to stabilise the analytes.

12 Discussion of the method

The method described herein allows for the determination of seven selenium species in urine: SeSug 1, SeSug 2, SeMet, SeEt, MeSeA, MeSeC, and MeSeG. The reversed-phase method is based on a method for the determination of various selenium species in human urine following the ingestion of nutrition supplements (Jäger et al. 2013). Using reversed-phase chromatography, all seven selenium species can be clearly separated. The applied procedure is characterised by minimal effort with respect to sample preparation, as the samples are only diluted with eluent 1 : 1 (v : v) and subsequently directly analysed. The limits of quantitation for the determination of the individual species lie between 0.18 µg/l and 0.34 µg/l urine (as selenium) and are thereby sufficient to measure exposure levels which exceed the background exposure of the general population.

Instruments used Agilent 1200 series HPLC system with a binary pump (G1312A) and an autosampler (G1329A) (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany) as well as Agilent 7500cx inductively coupled plasma-mass spectrometer with collision/reaction cell (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, 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.

References

Bader M, Barr D, Göen T, Schaller KH, Scherer G, Angerer J (2010) Reliability criteria for analytical methods. Biomonitoring Method, 2010. In: Angerer J, Hartwig A, editors. The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods. Volume 12. Weinheim: Wiley-VCH. p. 55–101. https://doi.org/10.1002/3527600418.bireliabe0012

Birringer M, Pilawa S, Flohé L (2002) Trends in selenium biochemistry. Nat Prod Rep 19(6): 693–718. https://doi.org/10.1039/B205802M

Bundesärztekammer (2014) Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. Dtsch Ärztebl 111(38): A1583–A1618

Butterman WC, Brown RD Jr (2004) Selenium. Mineral commodity profiles. Open-File Report 03–018. Reston: United States Geological Survey (USGS). https://pubs.usgs.gov/of/2003/of03-018/of03-018.pdf, accessed 28 Jan 2022

Cao TH, Cooney RA, Woznichak MM, May SW, Browner RF (2001) Speciation and identification of organoselenium metabolites in human urine using inductively coupled plasma mass spectrometry and tandem mass spectrometry. Anal Chem 73(13): 2898–2902. https://doi.org/10.1021/ac0100244

Combs GF Jr (2001) Selenium in global food systems. Br J Nutr 85(5): 517–547. https://doi.org/10.1079/BJN2000280

Cornelis R, Borguet F, De Kimpe J (1993) Trace elements in medicine. Speciation: the new frontier. Anal Chim Acta 283(1): 183–189. https://doi.org/10.1016/0003-2670(93)85221-5

D-A-CH (Deutsche Gesellschaft für Ernährung; Österreichische Gesellschaft für Ernährung; Schweizerische Gesellschaft für Ernährungsforschung; Schweizerische Vereinigung für Ernährung), editor (2016) Selen. In: Referenzwerte für die Nährstoffzufuhr, 2nd ed. Frankfurt am Main: Umschau Braus GmbH Verlagsgesellschaft

DFG (Deutsche Forschungsgemeinschaft), editor (2021) List of MAK and BAT Values 2021. Maximum Concentrations and Biological Tolerance Values at the Workplace. Permanent Senate Commission for the Investigation of Chemical Compounds in the Work Area, report 57. Düsseldorf: German Medical Science. https://doi.org/10.34865/mbwl_2021_eng

DIN (Deutsches Institut für Normung), editor (2008) DIN 32645:2008-11. Chemische Analytik – Nachweis-, Erfassungs- und Bestimmungsgrenze unter Wiederholbedingungen – Begriffe, Verfahren, Auswertung. Berlin: Beuth. https://doi.org/10.31030/1465413

ECHA (European Chemicals Agency) (2022) Substance infocard – Selenium. https://echa.europa.eu/de/substance-information/-/substanceinfo/100.029.052, accessed 28 Jan 2022

Eichler Š, Kaňa A, Kalousová M, Vosmanská M, Korotvička M, Zima T, Mestek O (2015) Speciation analysis of selenium in human urine by liquid chromatography and inductively coupled plasma mass spectrometry for monitoring of selenium in body fluids. Chem Speciation Bioavailability 27(3): 127–138. https://doi.org/10.1080/09542299.2015.1107502

Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE, Hurst R (2011) Selenium in human health and disease. Antioxid Redox Signal 14(7): 1337–1383. https://doi.org/10.1089/ars.2010.3275

Francesconi KA, Pannier F (2004) Selenium metabolites in urine: a critical overview of past work and current status. Clin Chem 50(12): 2240–2253. https://doi.org/10.1373/clinchem.2004.039875

Gammelgaard B, Jøns O (2000) Determination of selenite and selenate in human urine by ion chromatography and inductively coupled plasma mass spectrometry. J Anal At Spectrom 15(8): 945–949. https://doi.org/10.1039/B003637O

Gammelgaard B, Stürup S, Christensen MV (2012) Human urinary excretion and metabolism of ^{82}Se-enriched selenite and selenate determined by LC-ICP-MS. Metallomics 4(2): 149–155. https://doi.org/10.1039/c2mt00163b

Ganther HE (1986) Pathways of selenium metabolism including respiratory excretory products. J Am Coll Toxicol 5(1): 1–5. https://doi.org/10.3109/10915818609140731

Glover JR (1967) Selenium in human urine: a tentative maximum allowable concentration for industrial and rural populations. Ann Occup Hyg 10(1): 3–14. https://doi.org/10.1093/annhyg/10.1.3

Göen T, Greiner A (2018) Human biomonitoring of selenium exposure. In: Michalke B, editor. Selenium. Molecular and integrative toxicology. Cham: Springer International Publishing. p. 467–494. https://doi.org/10.1007/978-3-319-95390-8_24

Göen T, Schaller B, Jäger T, Bräu-Dümler C, Schaller KH, Drexler H (2015) Biological monitoring of exposure and effects in workers employed in a selenium-processing plant. Int Arch Occup Environ Health 88(5): 623–630. https://doi.org/10.1007/s00420-014-0989-7

Gómez-Ariza JL, Sánchez-Rodas D, Caro de la Torre MA, Giráldez I, Morales E (2000) Column-switching system for selenium speciation by coupling reversed-phase and ion-exchange high-performance liquid chromatography with microwave-assisted digestion–hydride generation–atomic fluorescence spectrometry. J Chromatogr A 889(1–2): 33–39. https://doi.org/10.1016/S0021-9673(00)00349-6

Greiner A, Hildebrand J, Feltes R, Uter W, Drexler H, Göen T (2020) Evaluation of urinary selenium as a biomarker of human occupational exposure to elemental and inorganic selenium. Int Arch Occup Environ Health 93(3): 325–335. https://doi.org/10.1007/s00420-019-01489-2

Greiner A, Drexler H, Hartwig A, MAK Commission (2021) Selenium and its inorganic compounds – Addendum for evaluation of BAR. Assessment Values in Biological Material – Translation of the German version from 2021. MAK Collect Occup Health Saf 6(3): Doc064. https://doi.org/10.34865/bb778249e6_3ad

Hartwig A, editor (2014) Selen und seine anorganischen Verbindungen. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 56th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb778249verd0056

Hartwig A, editor (2015) Selenium and its inorganic compounds. MAK Value Documentation, 2011. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Volume 26. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb778249vere5115

Hildebrand J, Greiner A, Drexler H, Göen T (2020) Determination of eleven small selenium species in human urine by chromatographic-coupled ICP-MS methods. J Trace Elem Med Biol 61: 126519. https://doi.org/10.1016/j.jtemb.2020.126519

Horng CJ, Tsai JL, Lin SR (1999) Determination of urinary arsenic, mercury, and selenium in steel production workers. Biol Trace Elem Res 70(1): 29–40. https://doi.org/10.1007/BF02783847

Jäger T, Drexler H, Göen T (2013) Ion pairing and ion exchange chromatography coupled to ICP-MS to determine selenium species in human urine. J Anal At Spectrom 28(9): 1402–1409. https://doi.org/10.1039/c3ja50083g

Jäger T, Drexler H, Göen T (2016) Human metabolism and renal excretion of selenium compounds after oral ingestion of sodium selenite and selenized yeast dependent on the trimethylselenium ion (TMSe) status. Arch Toxicol 90(5): 1069–1080. https://doi.org/10.1007/s00204-015-1548-z

Jiang S, Robberecht H, Vanden Berghe D (1983) Elimination of selenium compounds by mice through formation of different volatile selenides. Experientia 39(3): 293–294. https://doi.org/10.1007/BF01955311

Juresa D, Darrouzès J, Kienzl N, Bueno M, Pannier F, Potin-Gautier M, Francesconi KA, Kuehnelt D (2006) An HPLC/ICPMS study of the stability of selenosugars in human urine: implications for quantification, sample handling, and storage. J Anal At Spectrom 21: 684–690. https://doi.org/10.1039/b602976k

Kiss T, Odani A (2007) Demonstration of the importance of metal ion speciation in bioactive systems. Bull Chem Soc Jpn 80(9): 1691–1702. https://doi.org/10.1246/bcsj.80.1691

Kokarnig S, Tsirigotaki A, Wiesenhofer T, Lackner V, Francesconi KA, Pergantis SA, Kuehnelt D (2015) Concurrent quantitative HPLC–mass spectrometry profiling of small selenium species in human serum and urine after ingestion of selenium supplements. J Trace Elem Med Biol 29: 83–90. https://doi.org/10.1016/j.jtemb.2014.06.012

Kremer D, Ilgen G, Feldmann J (2005) GC–ICP–MS determination of dimethylselenide in human breath after ingestion of ^{77}Se-enriched selenite: monitoring of in-vivo methylation of selenium. Anal Bioanal Chem 383(3): 509–515. https://doi.org/10.1007/s00216-005-0001-1

Kuehnelt D, Kienzl N, Traar P, Le NH, Francesconi KA, Ochi T (2005) Selenium metabolites in human urine after ingestion of selenite, L-selenomethionine, or DL-selenomethionine: a quantitative case study by HPLC/ICPMS. Anal Bioanal Chem 383(2): 235–246. https://doi.org/10.1007/s00216-005-0007-8

Kuehnelt D, Juresa D, Kienzl N, Francesconi KA (2006) Marked individual variability in the levels of trimethylselenonium ion in human urine determined by HPLC/ICPMS and HPLC/vapor generation/ICPMS. Anal Bioanal Chem 386(7–8): 2207–2212. https://doi.org/10.1007/s00216-006-0848-9

Lajin B, Kuehnelt D, Jensen KB, Francesconi KA (2016) Investigating the intra-individual variability in the human metabolic profile of urinary selenium. J Trace Elem Med Biol 37: 31–36. https://doi.org/10.1016/j.jtemb.2016.06.008

Letsiou S, Nischwitz V, Traar P, Francesconi KA, Pergantis SA (2007) Determination of selenosugars in crude human urine using high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 21(3): 343–351. https://doi.org/10.1002/rcm.2843

Lu J, Holmgren A (2009) Selenoproteins. J Biol Chem 284(2): 723–727. https://doi.org/10.1074/jbc.R800045200

Lu Y, Rumpler A, Francesconi KA, Pergantis SA (2012) Quantitative selenium speciation in human urine by using liquid chromatography–electrospray tandem mass spectrometry. Anal Chim Acta 731: 49–59. https://doi.org/10.1016/j.aca.2012.04.016

Lund W (1990) Speciation analysis – why and how? Fresenius J Anal Chem 337(5): 557–564. https://doi.org/10.1007/BF00322862

Mao X, Hu B, He M, Chen B (2012) High polar organic–inorganic hybrid coating stir bar sorptive extraction combined with high performance liquid chromatography–inductively coupled plasma mass spectrometry for the speciation of seleno-amino acids and seleno-oligopeptides in biological samples. J Chromatogr A 1256: 32–39. https://doi.org/10.1016/j.chroma.2012.07.080

Marschall TA, Kroepfl N, Jensen KB, Bornhorst J, Meermann B, Kuehnelt D, Schwerdtle T (2017) Tracing cytotoxic effects of small organic Se species in human liver cells back to total cellular Se and Se metabolites. Metallomics 9(3): 268–277. https://doi.org/10.1039/C6MT00300A

Michalke B (2002 a) The coupling of LC to ICP-MS in element speciation – Part II: Recent trends in application. Trends Analyt Chem 21(3): 154–165. https://doi.org/10.1016/S0165-9936(02)00303-5

Michalke B (2002 b) The coupling of LC to ICP-MS in element speciation: I. General aspects. Trends Analyt Chem 21(2): 142–153. https://doi.org/10.1016/S0165-9936(01)00146-7

Navarro-Alarcon M, Cabrera-Vique C (2008) Selenium in food and the human body: a review. Sci Total Environ 400(1–3): 115–141. https://doi.org/10.1016/j.scitotenv.2008.06.024

Nuttall KL (2006) Evaluating selenium poisoning. Ann Clin Lab Sci 36(4): 409–420

Ogra Y, Hatano T, Ohmichi M, Suzuki KT (2003) Oxidative production of monomethylated selenium from the major urinary selenometabolite, selenosugar. J Anal At Spectrom 18(10): 1252–1255. https://doi.org/10.1039/b306710f

Ohta Y, Suzuki KT (2008) Methylation and demethylation of intermediates selenide and methylselenol in the metabolism of selenium. Toxicol Appl Pharmacol 226(2): 169–177. https://doi.org/10.1016/j.taap.2007.09.011

Rajotte BJP, P'an AYS, Malick A, Robin J-P (1996) Evaluation of selenium exposure in copper refinery workers. J Toxicol Environ Health 48(3): 239–251. https://doi.org/10.1080/009841096161311

Rayman MP (2000) The importance of selenium to human health. Lancet 356(9225): 233–241. https://doi.org/10.1016/S0140-6736(00)02490-9

Rayman MP (2012) Selenium and human health. Lancet 379(9822): 1256–1268. https://doi.org/10.1016/S0140-6736(11)61452-9

Rettenmeier AW (2019) Selenium and its inorganic compounds. BAT Value Documentation, 2011. MAK Collect Occup Health Saf. https://doi.org/10.1002/3527600418.bb778249vere1819

RKI (Robert Koch-Institut) (2006) Selen in der Umweltmedizin: Mitteilung der Kommission "Methoden und Qualitätssicherung in der Umweltmedizin". Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 49(1): 88–101. https://doi.org/10.1007/s00103-005-1185-4

RÖMPP-Redaktion, Hartwig A (2006) Selen. https://roempp.thieme.de/lexicon/RD-19-01826, accessed 21 Feb 2022

SCF (Scientific Committee on Food) (2006) Tolerable upper intake levels for vitamins and minerals. Parma: EFSA. https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf, accessed 28 Jan 2022

WHO (World Health Organization) (1987) Selenium. Environmental health criteria, No. 58. Geneva: WHO. https://apps.who.int/iris/bitstream/handle/10665/37268/9241542586-eng.pdf?sequence=1%26isAllowed=y, accessed 21 Feb 2022

Zheng J, Ohata M, Furuta N (2002) Reversed-phase liquid chromatography with mixed ion-pair reagents coupled with ICP-MS for the direct speciation analysis of selenium compounds in human urine. J Anal At Spectrom 17(7): 730–735. https://doi.org/10.1039/b202531k