The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Selenium and its compounds – Determination of selenosugar 3 and trimethylselenonium in urine by cation-exchange chromatography-ICP-MS

Biomonitoring Method – Translation of the German version from 2022

Keywords

selenium species; speciation analysis; biomonitoring; urine; cation-exchange chromatography; HPLC; ICP-MS

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)*

Citation Note:
Hildebrand J, Jäger T, Göen T, Michalke B, Hartwig A, MAK Commission. Selenium and its compounds – Determination of selenosugar 3 and trimethylselenonium in urine by cation-exchange chromatography-ICP-MS. Biomonitoring Method – Translation of the German version from 2022. MAK Collect Occup Health Saf. 2022 Sep;7(3):Doc069. https://doi.org/10.34865/bi778249ce7_3or

Manuscript completed:
11 Mar 2021

Publication date:
30 Sep 2022

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 two organic selenium species with cationic character in urine. Selenosugar 3 (SeSug 3) and trimethylselenonium (TMSe) can be separated by cation-exchange chromatography and subsequently detected using inductively coupled plasma-mass spectrometry (ICP-MS). Calibration is performed using standard solutions in pooled urine, which are processed and analysed analogously to the samples.

1 Characteristics of the method

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

Parameters and corresponding hazardous substances

Hazardous substances	CAS No.	Parameter	CAS No.
Selenium and its compounds	7782-49-2	Methyl-2-amino-2-deoxy-1-seleno-*β*-*D*-galactopyranoside (Selenosugar 3, SeSug 3)	866430-57-1
		Trimethylselenonium (TMSe)	25930-79-4

Reliability criteria

Selenosugar 3 (SeSug 3)

Within-day precision:	Standard deviation (rel.)	s_w = 11.3%
	Prognostic range	u = 25.6%
	at a spiked concentration of 2.2 µg per litre of urine (as selenium) and n = 10 determinations	
Day-to-day precision[a)]:	Standard deviation (rel.)	s_w = 10.8%
	Prognostic range	u = 27.8%
	at a spiked concentration of 2.5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy[a)]:	Recovery rate (rel.)	r = 93.9%
	at a spiked concentration of 2.5 µg per litre of urine (as selenium) and n = 6 determinations	
Detection limit:	0.10 µg per litre of urine (as selenium)	
Quantitation limit:	0.28 µg per litre of urine (as selenium)	

[a)] Data were collected by the external verifier of the method.

Trimethylselenonium (TMSe)

Within-day precision:	Standard deviation (rel.)	s_w = 9.4%
	Prognostic range	u = 21.3%
	at a spiked concentration of 2.9 µg per litre of urine (as selenium) and n = 10 determinations	
Day-to-day precision[a)]:	Standard deviation (rel.)	s_w = 6.4%
	Prognostic range	u = 16.5%
	at a spiked concentration of 2.5 µg per litre of urine (as selenium) and n = 6 determinations	
Accuracy[a)]:	Recovery rate (rel.)	r = 100%
	at a spiked concentration of 2.5 µg per litre of urine (as selenium) and n = 6 determinations	
Detection limit:	0.10 µg per litre of urine (as selenium)	
Quantitation limit:	0.28 µg per litre of urine (as selenium)	

[a)] Data were collected by the external verifier of the method.

2 General information on selenium

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).

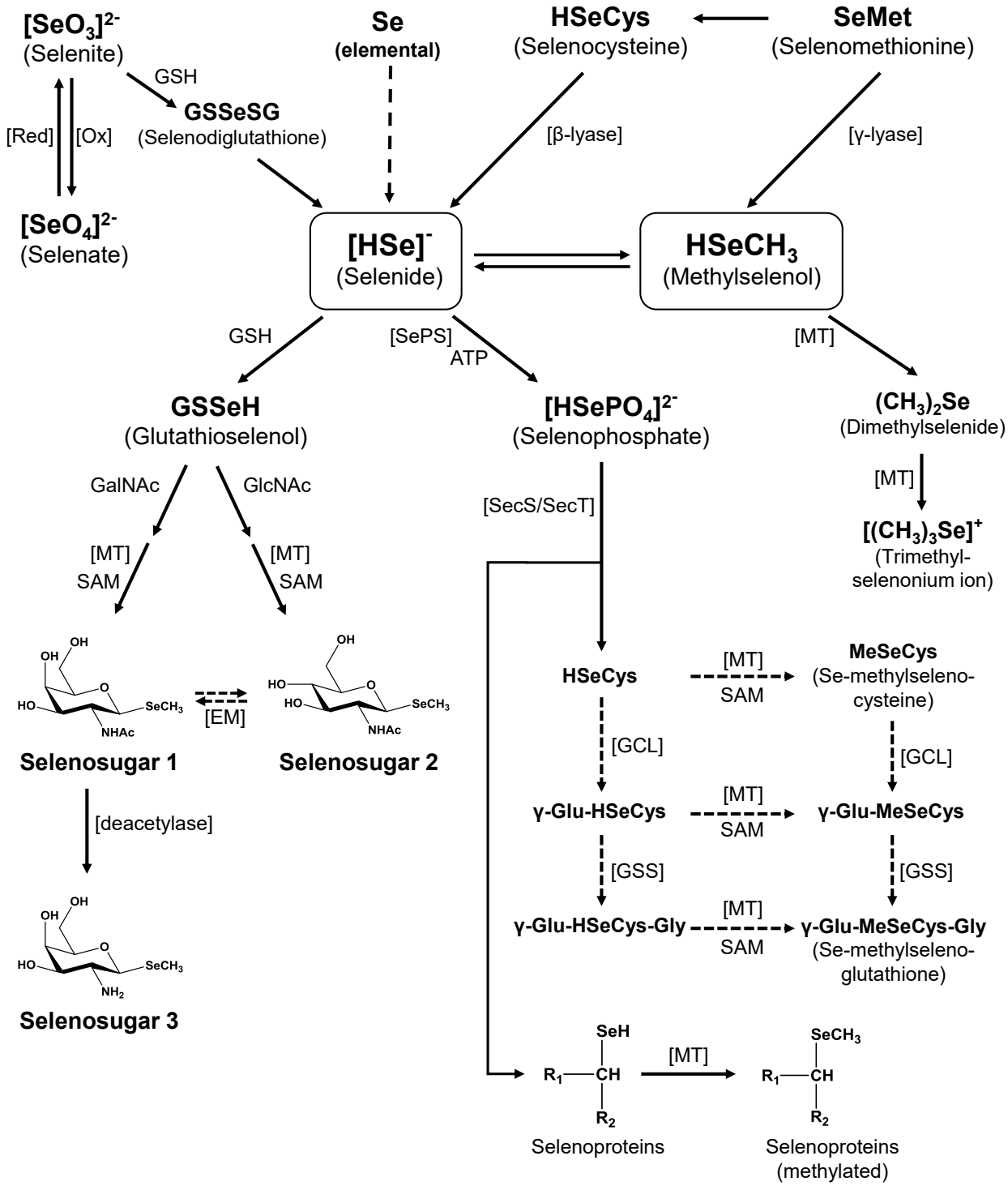
Tab. 1 Selenium concentrations in urine following occupational exposure

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
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 2022; 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 value, 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).



EM: epimerase; GalNAc: *N*-acetylgalactosamine; GCL: glutamate-cysteine ligase; GlcNAc: *N*-acetylglucosamine; GSH: glutathione; GSS: glutathione synthetase; MT: methyltransferase; SAM: *S*-adenosyl methionine; SecS/SecT: *L*-seryl-tRNASec selenium transferase; SePS: selenophosphate synthetase

Fig. 1 Metabolism scheme of selenium according to Navarro-Alarcon and Cabrera-Vique (2008) as well as Fairweather-Tait et al. (2011)

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.

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.

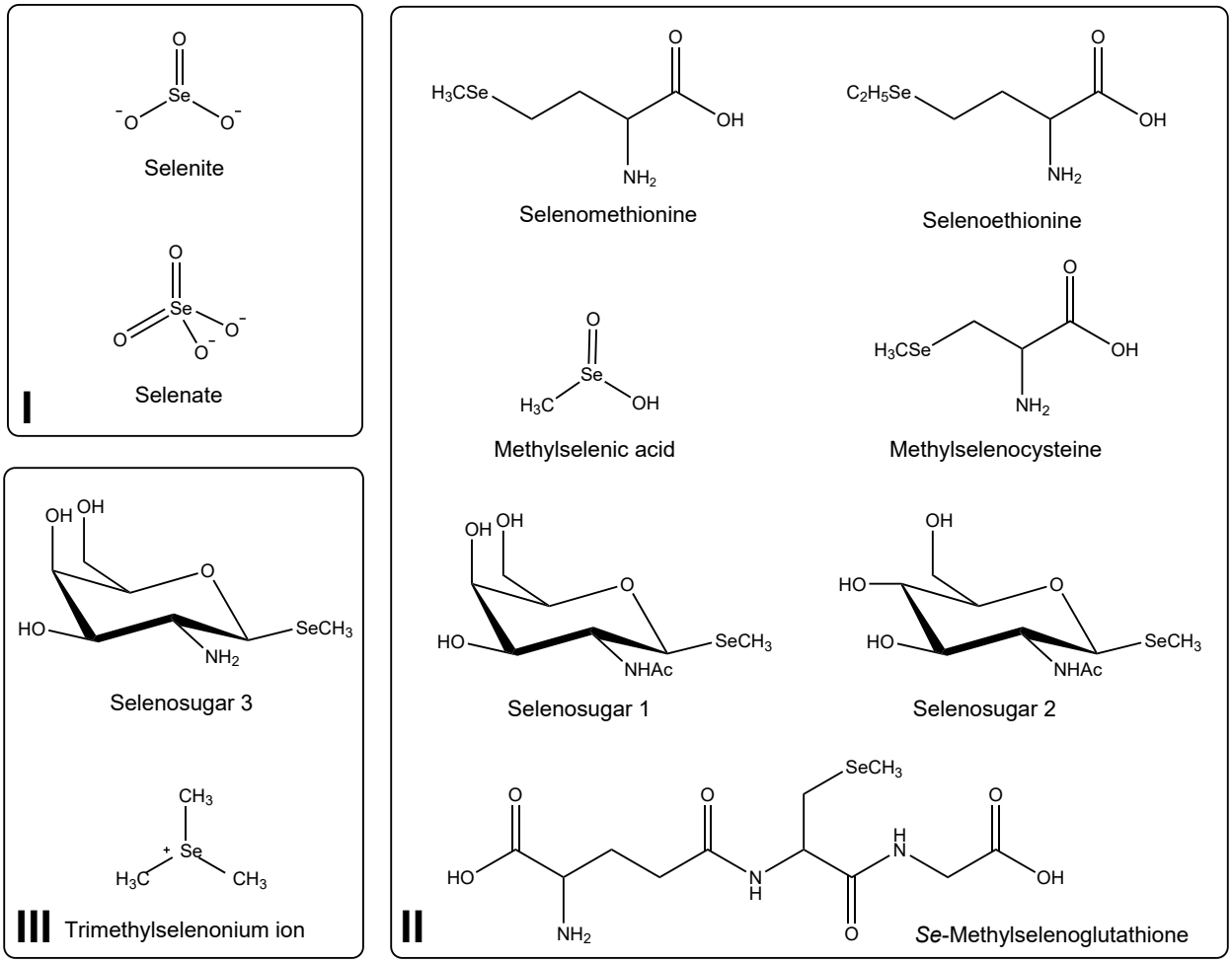


Fig. 2 Structures of the selenium species which can be determined with the three submethods

The determination of SeSug 3 and TMSe is carried out by cation-exchange chromatography and is described in the hereby presented Submethod III. There are no published data on the urinary concentrations of SeSug 3 and TMSe following occupational exposure. The published data on urine levels of these species have been determined in the non-occupationally exposed general population or after supplementation using selenium-containing preparations.

The formation of TMSe is subject to large interindividual variations, whereby "TMSe eliminators" are distinguished from "non-TMSe eliminators." The background levels for non-eliminators generally lie below the limit of detection

of the corresponding analytical method (Jäger et al. 2013, 2016; Lu et al. 2012); otherwise, very low concentrations were observed in the ranges of < LOD–0.2 µg Se/l (Lajin et al. 2016), < LOD–0.09 µg Se/l (Kuehnelt et al. 2006), or 0.092 ± 0.089 µg Se/l (Kuehnelt et al. 2015). In non-eliminators, no increase in TMSe concentration can be observed following selenium supplementation (Jäger et al. 2016; Kuehnelt et al. 2006; Lajin et al. 2016; Lu et al. 2012). In contrast, considerably higher background levels were observed in TMSe eliminators. Moreover, in TMSe eliminators, urinary TMSe levels increase substantially following selenium supplementation (see Table 2).

Tab. 2 TMSe concentrations in the urine of TMSe eliminators (background levels or following selenium supplementation)

Collective (sample number n; quantity > LOD[a])	TMSe		References
	Background concentration	After supplementation	
General population, Germany (47; 8)	2.11 µg Se/l[b] (1.12–4.95 µg Se/l)	–	Jäger et al. 2013
General population, USA (13; 9)	11–63 µg Se/l	–	Blotcky et al. 1987
General population, Spain (3)	14.3 – 19.8 µg Se/l	–	Quijano et al. 1999
General population (women), Bangladesh (75) and Argentina (3)	1.9 ± 0.87 µg Se/l to 3.2 ± 0.59 µg Se/l	–	Kuehnelt et al. 2015
General population, Taiwan (4; 2)	11–13.2 µg Se/l	–	Yang and Jiang 1995
General population, Japan (9)	5.4–26.5 µg Se/l	–	Zheng et al. 2002
Selenium supplementation, 200 µg Se as selenite (9; 3) or 100 µg Se as selenium yeast (7; 3), Germany	2.9 ± 1.1 µg Se/24 h or 2.6 ± 0.5 µg Se/24 h	9.6 ± 3.9 µg Se/24 h or 5.3 ± 2.8 µg Se/24 h	Jäger et al. 2016
Selenium supplementation, 200 µg Se as selenite, followed by 400 µg Se as selenite, Austria (8; 2)	4.4 µg Se/l (3.2–6.2 µg Se/l)[c]	8.4 µg Se/l (5.7–13 µg Se/l) or 15.9 µg Se/l (7.9–24.8 µg Se/l)[c]	Lajin et al. 2016
Selenium supplementation, 200 µg Se as selenite, Austria (7;2)	0.18–0.37 µg Se/l	4.6–15 µg Se/l	Kuehnelt et al. 2006
Selenium supplementation, 200 µg Se as selenate or 200 µg Se as selenomethionine, New Zealand (26)	0.3–0.9 µg Se/24 h	1.7–2.3 µg Se/24 h or 0.9–1.9 µg Se/24 h	Robinson et al. 1997
Selenium supplementation, 200 µg Se as selenomethionine, Greece (2; 1)	< LOD–0.79 µg Se/l	< LOD–10.2 µg Se/l	Lu et al. 2012

a) LOD: limit of detection
b) Median (range)
c) Mean (range)

Background levels of SeSug 3 have been investigated in several studies. In the German general population, concentrations have been found in the range of < LOD–2.41 µg Se/l (median: 0.74 µg Se/l; n = 47) (Jäger et al. 2013). These concentrations correspond with the values published by Kuehnelt et al. (2006) (< LOD–1.7 µg Se/l; n = 7), while Lajin et al. (2016) observed slightly higher concentrations (median: 2.3 µg Se/l; range: 0.8–4.1 µg Se/l; n = 8). After supplementation with 200 µg selenium as selenite, SeSug 3 excretion increased to 1.0–9.1 µg Se/l (median: 3.9 µg Se/l); after supplementation with 400 µg selenium as selenite, it increased to 2.7–16.7 µg Se/l (median: 9.0 µg Se/l) (Lajin et al. 2016). After supplementation with 200 µg selenium as selenite, Kuehnelt et al. (2006) likewise observed an increase in SeSug 3 concentrations and reported values of 0.9–3.7 µg Se/l. Before supplementation with 200 µg selenium as selenite or 100 µg selenium as selenium yeast, Jäger et al. (2016) found SeSug 3 levels in the range of 1.7 ± 1.0 µg Se/24 h or 1.3 ± 0.7 µg Se/24 h. Following supplementation, concentrations were slightly higher, with values in the range of 2.1 ± 0.9 Se/24 h or 1.7 ± 0.9 Se/24 h.

3 General principles

The analytical method described herein is used to determine two organic selenium species with cationic character in urine. SeSug 3 and TMSe are separated by cation-exchange chromatography and subsequently quantified by 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 (e.g. Shodex RSpak NN-614 (150 × 6 mm; 10 µm) with Shodex RSpak NN-G guard column (50 × 6.0 mm; 10 µm), Showa Denko Europe GmbH, Munich, Germany)
- Ultrasonic bath (e.g. VWR International GmbH, Darmstadt, Germany)
- Vortex mixer (e.g. Heidolph Instruments GmbH & Co. KG, Schwabach, Germany)
- Centrifuge (e.g. Fisher Scientific GmbH, Schwerte, Germany)
- pH meter (e.g. VWR International GmbH, Darmstadt, Germany)
- 13-ml polypropylene tubes with caps (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)
- Precision balance (e.g. Mettler-Toledo GmbH, Gießen, Germany)
- Cellulose filters, pore size of 0.45 µm, membrane of mixed cellulose esters (e.g. Merck KGaA, Darmstadt, Germany)
- 1.5-ml reaction vessels (e.g. Eppendorf AG, Hamburg, Germany)
- 250-ml and 1000-ml beakers (e.g. VWR International GmbH, Darmstadt, Germany)
- 100-ml laboratory glass bottle (e.g. VWR International GmbH, Darmstadt, Germany)
- 10-, 100- and 1000-ml volumetric flasks (e.g. VWR International GmbH, Darmstadt, Germany)
- 1.5-ml amber glass vials (e.g. VWR International GmbH, Darmstadt, Germany)
- Urine cups (e.g. Sarstedt AG & Co. KG, Nümbrecht, Germany)
- Various pipettes 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.

- Ammonium dihydrogen phosphate (e.g. No. 1.01126, Merck KGaA, Darmstadt, Germany)
- Sodium hydroxide pellets (e.g. No. 1.06469, Merck KGaA, Darmstadt, Germany)
- Ethanol (e.g. No. 1.00983, Merck KGaA, Darmstadt, Germany)
- Ultra-pure water (e.g. Milli-Q® Direct Water Purification System, Merck KGaA, Darmstadt, Germany)

- Pooled urine from individuals who are neither occupationally exposed to selenium nor ingest selenium via nutritional supplements, refrigerated for 48 h
- Argon 5.0 (e.g. Linde GmbH, Pullach, Germany)
- Hydrogen 5.0 (e.g. Linde GmbH, Pullach, Germany)

4.3 Reference standards

- Methyl-2-amino-2-deoxy-1-seleno-*β*-*D*-galactopyranoside (SeSug 3); purity: > 95% (custom synthesis, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany)
- Trimethylselenonium iodide (synthesis according to Hoffman (1991), Institute of Occupational, Social, and Environmental Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, 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

- Ammonium dihydrogen phosphate solution (1 mol/l)
  11.5 g of ammonium dihydrogen phosphate are weighed into a 100-ml volumetric flask and dissolved in ultra-pure water. The flask is then made up to the mark with ultra-pure water.
- Sodium hydroxide solution (1 mol/l)
  90 ml of ultra-pure water are placed in a 250-ml beaker. 4.0 g of sodium hydroxide pellets are carefully added and dissolved while stirring. The sodium hydroxide solution is transferred into a 100-ml volumetric flask, which is then made up to the mark with ultra-pure water. The solution is stored in a screw-top laboratory glass bottle.
- Ammonium dihydrogen phosphate solution (30 mmol/l) with 1% ethanol (pH = 6.3; Eluent A)
  900 ml of ultra-pure water are placed in a 1000-ml beaker. Subsequently, 30 ml of the ammonium dihydrogen phosphate solution (1 mol/l) as well as 10 ml of ethanol are added. Using the sodium hydroxide solution (1 mol/l), the pH value is adjusted to pH = 6.3, and the solution is transferred into a 1000-ml volumetric flask. The flask is made up to the mark with ultra-pure water.
  Eluent A is prepared freshly for each analysis, filtered prior to use, and degassed in an ultrasonic bath for five minutes.
- Ammonium dihydrogen phosphate solution (0.5 mmol/l) with 1% ethanol (pH = 6.3; Eluent B)
  900 ml of ultra-pure water are placed in a 1000-ml beaker. Subsequently, 0.5 ml of the ammonium dihydrogen phosphate solution (1 mol/l) as well as 10 ml of ethanol are added. Using the sodium hydroxide solution (1 mol/l), the pH value is adjusted to pH = 6.3, and the solution is transferred into a 1000-ml volumetric flask. The flask is made up to the mark with ultra-pure water.
  Eluent B is prepared freshly 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)
  33 mg of SeSug 3 (257.2 g/mol) or 32 mg of TMSe iodide (251.0 g/mol) are each weighed into a 10-ml volumetric flask. The flasks are then made up to the mark with ultra-pure water.

Calculated as selenium, the solutions thus prepared contain about 1 g Se/l. In order to determine the exact selenium concentrations, the stock solutions are measured against an ICP single-element standard in order to correct for any potential weighing errors.

- 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 100-ml volumetric flasks. 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 reaction vessel. Subsequently, 800 µl of ultra-pure water are added by pipetting, 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 reaction vessel with 900 µl of ultra-pure water and mixed.

The stock and working solutions of the analytes are stored in polypropylene tubes at –18 °C.

For the preparation of 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 prepared freshly for each analytical run.

As the slopes of the calibration curves in eluent differ from those in pooled urine, the calibration standards must be prepared in urine for the correct quantification of the relevant selenium species.

Tab. 3 Pipetting scheme for the preparation of calibration standards for the determination of SeSug 3 and TMSe in urine

Calibration standard	Spiking solution I [µl]	Spiking solution II [µl]	Pooled urine [ml]	Concentration [µg Se/l]
0	–	–		0
1	–	20		0.20
2	–	50		0.50
3	–	100	ad 10	1.0
4	–	200		2.0
5	50	–		5.0
6	100	–		10.0
7	250	–		25.0

5 Specimen collection and sample preparation

5.1 Specimen collection

The urine samples are collected in urine cups. Following specimen collection, the urine samples should be processed and analysed as soon as possible. See Section 11.4 for questions regarding the storage stability of SeSug 3.

5.2 Sample preparation

The urine samples are brought to room temperature and thoroughly mixed. In a 1.5-ml reaction vessel, 900 µl of the sample are mixed with 100 µl of eluent A (ammonium dihydrogen phosphate solution (30 mmol/l) with 1% ethanol (pH = 6.3)) and subsequently thoroughly mixed for ten seconds. Of the sample thus diluted, 50 µl are applied for analysis.

6 Operational parameters

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

6.1 Liquid chromatography

Analytical column:	Shodex RSpak NN-614 (150 × 6 mm; 10 µm) with Shodex RSpak NN-G guard column (50 × 6.0 mm; 10 µm)
Separation principle:	Cation-exchange chromatography
Column temperature:	50 °C
Eluent A:	Ammonium dihydrogen phosphate (30 mmol/l) with 1% ethanol (pH = 6.3)
Eluent B:	Ammonium dihydrogen phosphate (0.5 mmol/l) with 1% ethanol (pH = 6.3)
Flow rate:	0–14.5 min: 0.8 ml/min Eluent A
	14.5–19.5 min: 0.8 ml/min Eluent B
	19.5–20 min: 0.8 ml/min Eluent A
Injection volume:	50 µl
Runtime:	20 min

The parameters given above must be optimised according to manufacturer specifications.

6.2 Inductively coupled plasma-mass spectrometry

Rf power:	1500 W
Nebuliser:	MikroMist
Spray chamber:	Scott Quarz
Spray-chamber temperature:	20 °C
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:	1.0 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 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. The selected mass traces as well as the retention times are summarised in Table 4. The mass trace ^{78}Se is used for quantitation.

Tab. 4 Retention times and parameter-specific settings for the determination of SeSug 3 and TMSe in urine

Analyte	Retention time [min]	Analysed mass trace	Measuring time per mass trace [s]
SeSug 3	8.5	^{78}Se	6
TMSe	12.0	^{78}Se	6

7 Analytical determination

Of each of the samples processed according to Section 5.2, 50 µl are injected into the HPLC-ICP-MS system. The analytes SeSug 3 and TMSe are identified by their retention times. The retention times given in Table 4 can only serve as a point of reference. The user must ensure proper separation performance of the HPLC column used and the resulting retention behaviour of the analytes. Figure 3 shows a representative chromatogram of a urine sample spiked with SeSug 3 and TMSe.

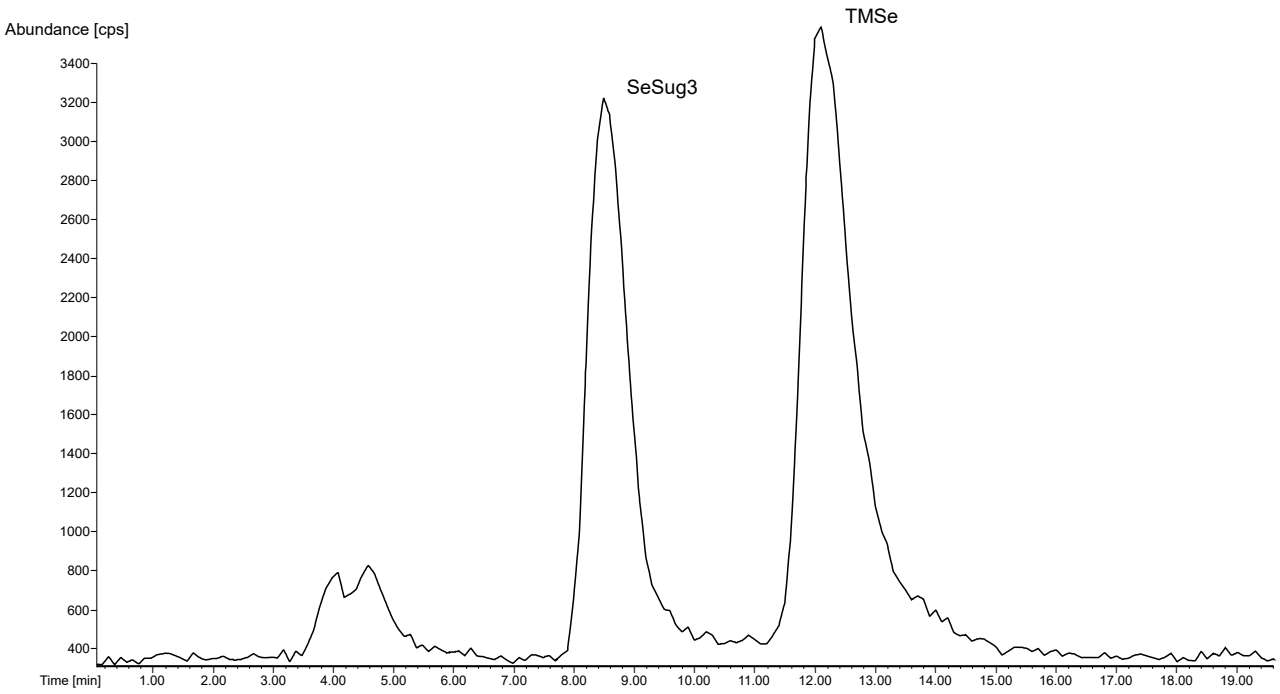


Fig. 3 Chromatogram of a urine sample spiked with SeSug 3 (25 µg Se/l) and TMSe (25 µg Se/l)

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 concentrations of the corresponding calibration standards. The calibration curves are linear under the described conditions from 0.25 to 25.0 µg/l (as selenium). Figure 4 shows representative calibration curves for the determination of SeSug 3 and TMSe in urine.

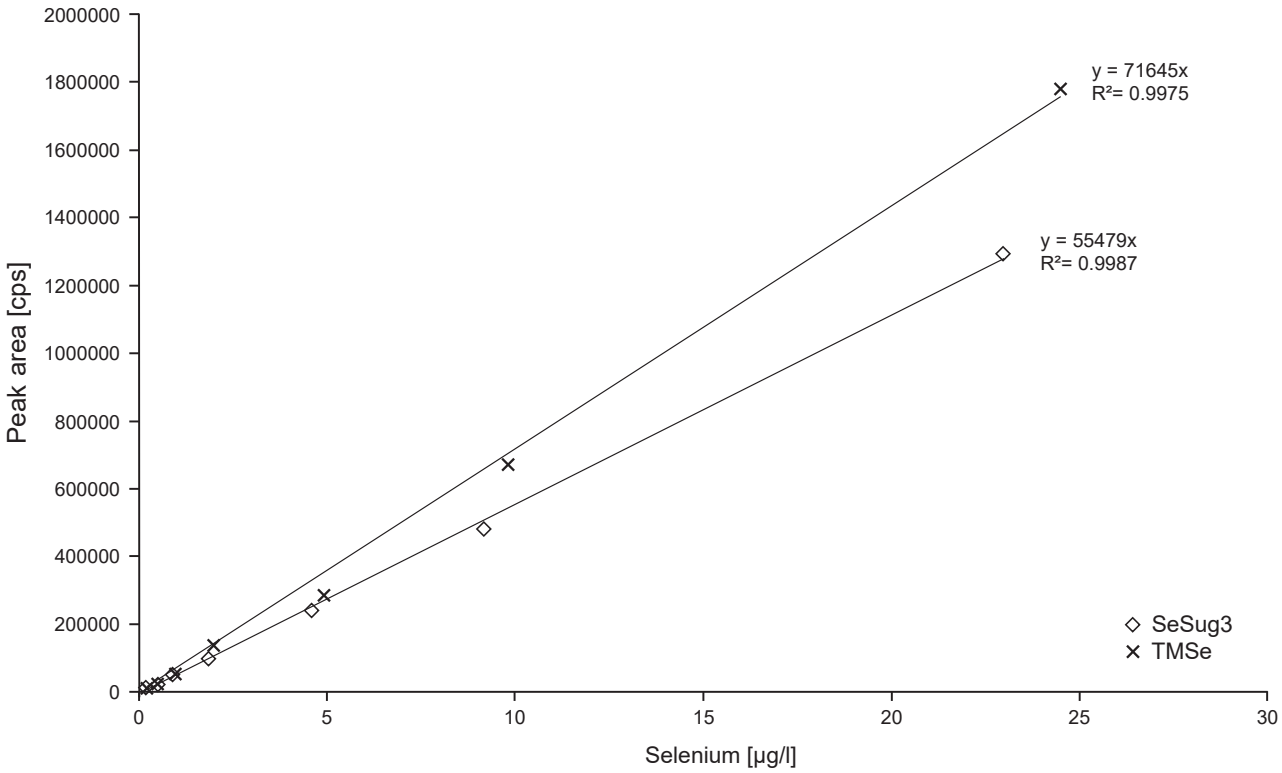


Fig. 4 Calibration curves for the determination of SeSug 3 and TMSe in urine

9 Calculation of the analytical results

The analyte concentration of a sample in µg/l urine (as selenium) can be calculated by entering the peak area of the analyte in question into the calibration function for the corresponding analytical run. The calibration range may need to be adjusted to the expected concentration range. If the result lies above the calibration range, the sample 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).

To ensure the quality of the analytical results, two quality-control samples with different analyte concentrations are processed parallel to the samples and analysed as part of each analytical run. Since control materials are not currently commercially available, the material must be prepared in the in-house laboratory. For this purpose, pooled urine from individuals who are neither occupationally exposed to selenium nor ingest selenium via nutritional supplements is spiked with both selenium species. The spiked selenium levels must lay within the expected concentration range. The prepared quality-control materials are each aliquoted to 900 µl in 1.5-ml reaction vessels and stored at –18 °C until analysis.

The nominal value and the tolerance range (mean ± two standard deviations) of each quality-control material are determined during a pre-analytical period (Bader et al. 2010).

In addition, a reagent blank consisting of 1 ml of ultra-pure water is included in each analytical run.

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, pooled urine was spiked with SeSug 3 and TMSe, at a concentration of 2.2 µg/l and 2.9 µg/l (as selenium), respectively. The material was processed and analysed ten 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 SeSug 3 and TMSe in urine (n = 10)

Analyte	Spiked concentration [µg Se/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
SeSug 3	2.2	11.3	25.6
TMSe	2.9	9.4	21.3

Day-to-day precision

Day-to-day precision for the determination of SeSug 3 and TMSe was investigated by the external verifier of the method. To this end, pooled urine was spiked with 2.5 µg/l (as selenium) of each analyte, quantified, 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 SeSug 3 and TMSe in urine (n = 6)

Analyte	Spiked concentration [µg Se/l]	Standard deviation (rel.) s_w [%]	Prognostic range u [%]
SeSug 3	2.5	10.8	27.8
TMSe	2.5	6.4	16.5

11.2 Accuracy

The accuracy of the method was investigated by the external verifier of the method. To this end, six aliquots of a urine sample were each spiked with 2.5 µg/l (as selenium) of each analyte. The unspiked urines were likewise processed and analysed. Relative recovery was calculated using the determined concentrations of the spiked urine, deducting any background levels which arose in the unspiked urines. The relative recovery rates thus obtained are given in Table 7.

Tab. 7 Relative recovery rates for the determination of SeSug 3 and TMSe in urine (n = 6)

Analyte	Spiked concentration [µg Se/l]	Mean rel. recovery r [%]
SeSug 3	2.5	93.9
TMSe	2.5	100

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 determination of SeSug 3 and TMSe in urine.

Tab. 8 Limits of detection and quantitation for the determination of SeSug 3 and TMSe in urine (n = 3)

Analyte	Detection limit [µg Se/l]	Quantitation limit [µg Se/l]
SeSug 3	0.10	0.28
TMSe	0.10	0.28

11.4 Sources of error

In determining day-to-day precision for SeSug 3, the developers of this method found a higher scattering of the measured values compared with the external verifier of the method. It could not be conclusively resolved whether the worse precision data could be explained by the degradation of SeSug 3. In order to avoid potential SeSug 3 degradation, the urines samples should be processed and analysed as quickly as possible following specimen collection.

If 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). Since SeSug 1 and SeSug 2 in urine samples can be stabilised by pH-value adjustment and the addition of sodium azide (pH = 5.5; 0.1% sodium azide) (Hildebrand et al. 2020), SeSug 3 may potentially be stabilised in the same manner. For the stabilisation of SeSug 1 and SeSug 2, Juresa et al. (2006) successfully tested the storage of urines at −80 °C and the storage of lyophilised urine samples at −20 °C. Furthermore, the authors recommended the addition of sodium azide or bubbling of nitrogen through the samples.

12 Discussion of the method

The submethod described herein serves the determination of two cationic selenium species in urine: SeSug 3 and TMSe. This method is based on a method for the determination of TMSe in urine by Kuehnelt et al. (2006). In the eluent, SeSug 3 and TMSe can be separated from the remaining selenium species using cation-exchange chromatography. The quantitation limits of both species lie at 0.28 µg/l (as selenium) and are thereby largely sufficient to determine even background levels in the general population.

The external verifier of the method could easily replicate the method, even when using a different column (Hamilton PRP X 200 PEEK (250 × 4.6 mm; 10 µm) with Hamilton PRP X 200 guard column (20 × 2 mm; 10 µm)), although the analyte retention times clearly shifted and got shorter.

In cases of sufficient sensitivity, the sample volume may be reduced from 50 µl to 25 µl in order to prolong the lifetime of the analytical column.

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

Blotcky AJ, Hansen GT, Borkar N, Ebrahim A, Rack EP (1987) Simultaneous determination of selenite and trimethylselenonium ions in urine by anion exchange chromatography and molecular neutron activation analysis. Anal Chem 59(17): 2063–2066. https://doi.org/10.1021/ac00144a012

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

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 (2022) List of MAK and BAT Values 2022. Maximum Concentrations and Biological Tolerance Values at the Workplace. Permanent Senate Commission for the Investigation of Chemical Compounds in the Work Area, report 58. Düsseldorf: German Medical Science. https://doi.org/10.34865/mbwl_2022_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

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

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

Hoffman JL (1991) Ion chromatographic analysis of the purity and synthesis of sulfonium and selenonium ions. J Chromatogr A 588(1–2): 211–216. https://doi.org/10.1016/0021-9673(91)85025-B

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

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

Kuehnelt D, Engström K, Skröder H, Kokarnig S, Schlebusch C, Kippler M, Alhamdow A, Nermell B, Francesconi K, Broberg K, Vahter M (2015) Selenium metabolism to the trimethylselenonium ion (TMSe) varies markedly because of polymorphisms in the indolethylamine N-methyltransferase gene. Am J Clin Nutr 102(6): 1406–1415. https://doi.org/10.3945/ajcn.115.114157

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

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

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

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

Quijano MA, Gutiérrez AM, Pérez-Conde MC, Cámara C (1999) Determination of selenium species in human urine by high performance liquid chromatography and inductively coupled plasma mass spectrometry. Talanta 50(1): 165–173. https://doi.org/10.1016/S0039-9140(99)00115-0

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

Robinson MF, Thomson CD, Jenkinson CP, Luzhen G, Whanger PD (1997) Long-term supplementation with selenate and selenomethionine: urinary excretion by New Zealand women. Br J Nutr 77(4): 551–563. https://doi.org/10.1079/BJN19970056

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

Yang K-L, Jiang S-J (1995) Determination of selenium compounds in urine samples by liquid chromatography-inductively coupled plasma mass spectrometry with an ultrasonic nebulizer. Anal Chim Acta 307(1): 109–115. https://doi.org/10.1016/0003-2670(95)00010-W

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