Tanja Wiesenhofer, BSc

Direct determination of selenium in biological fluids

Determination of total selenium in human urine by flow injection coupled to inductively coupled plasma mass spectrometry

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Ass.-Prof. Mag. Dr.rer.nat. Doris Kühnelt

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Contents

1 Summary

Selenium is an essential trace element, which has many biochemical functions but also shows toxic effects at elevated intake. Therefore, it is important to monitor the selenium body status e.g. by investigating total selenium in biological fluids like blood or urine. Inductively coupled plasma mass spectrometry (ICPMS) is a robust technique with low limits of detection for the determination of selenium in body fluids. Low sample volumes can be investigated directly, without time-consuming digestion or dilution, when flow injection (FI) is used for sample introduction.

The aim of this work was to develop a FI/ICPMS method for the determination of total selenium in biological fluids requiring minimum sample preparation. Quantification should be based on external calibration to avoid time-consuming standard addition approaches. FI/ICPMS signals were optimized regarding carrier solutions and flow rates. Matrix effects occurring when urine is analyzed directly without digestion or dilution were minimized by splitting the flow of the carrier solution, by optimizing the injection volume, and by using germanium as internal standard. Best results were obtained with 20 mM malonate 3 % MeOH pH 6.0 containing 50 µg Ge/L as carrier solution at a flow rate of 1.5 mL/min, whereby 15 % of the carrier solution were directed to the ICPMS by a passive splitter, and an injection volume of 5 µL. The method detection and quantification limits in urine were 0.2 µg Se/L and 0.8 µg Se/L, respectively. The accuracy of the method was evaluated by (i) the certified reference material NIES No. 18 human urine, (ii) comparison of FI/ICPMS results for different urine samples with results obtained by ICPMS after microwave-assisted acid digestion, and (iii) by spiking recovery experiments. Intra-day precision for the determination of total selenium in urine was about 3 % and inter-day precision about 5 % depending on the concentration. Urine samples could be stored for at least 4 days at 4 °C before volatile compounds known to lead to overestimated results were formed in the urine. Simple sample preparation by filtration together with an analysis time of about 2 minutes/sample, as well as the low requirement for sample volume makes the method very useful particularly for large numbers of samples and samples for which only limited volumes are available.

2 Introduction

2.1 Selenium

2.1.1 History

In 1817 selenium was isolated for the first time by the Swedish scientist Jöns Jakob Berzelius. He noted the similarity of the new element to the earlier-discovered tellurium (tellus is the latin name of the earth). Therefore, he named selenium after the Greek word for moon (Σελήνη = Selene). [1] After 1817, indication for selenium toxicity came up, since horses were suffering from a deadly disease after grazing in certain regions of China and the USA, [2] where the grains contained very high amounts of selenium. [3] In 1957, evidence was gathered that selenium is an essential trace element for humans. [4] Hence, selenium is essential for humans in low concentrations, but has toxic effects at higher concentrations, with a very narrow range between selenium deficiency, essentiality, and toxicity. [3]

2.1.2 Chemical and physical properties

The atomic weight of selenium is 78.96 and its atomic number is 34. It is located in group 16 between sulfur and tellurium and in period 4 between arsenic and bromine in the periodic table of the elements. Therefore, it is placed in the group of metalloids. The compounds of selenium show similar behavior as those of its neighbors. [5]

Selenium commonly occurs in four oxidation states: -2 (selenide), 0 (elemental selenium), +4 (selenite) and +6 (selenate) [6] and has six stable isotopes: ^{74}Se (0.89 %), ^{76}Se (9.36 %), ^{77}Se (7.63 %), ^{78}Se (23.78 %), ^{80}Se (49.61 %) and ^{82}Se (8.73 %). [7] In its elemental form, selenium is very stable and highly insoluble. Soluble selenium compounds occuring in soils, such as selenates, become unavailable for absorption by plants under reducing conditions, since these compounds are converted to elemental selenium. [1]

Selenium has specific electrical properties, which are responsible for its usefulness to electrical and electronic industries. Its conductivity is low in the dark, but is increased several 100-fold when it is exposed to light. Then it also generates a small electrical current in the element and, hence, has the properties of a semiconductor. [1]

2.1.3 Occurrence

Because of the uneven geographical distribution of selenium, its concentration in biological materials depends on geological factors. [8] Due to its similarity to sulfur with respect to bond

energy, ionization potential, electron affinity and atomic radius, selenium is preferentially located in materials which contain sulfur in high concentrations. [9, 10]

Selenium is transferred up the soil-plant-animal-human food chain. [11] Plants take up selenium from the soil primarily as selenate or selenite, which are then metabolized to selenomethionine (for structures see Figure 1). This organic form of selenium is bioavailable to animals and humans. [12] Geographical differences in soil selenium concentrations lead to variations in the selenium content of food. [11] The bioavailability of selenium to plants also depends on the pH of the soil and the presence of sulfur, which can compete with selenium for absorption. In addition, the amount of organic matter, iron hydroxides and aluminium compounds in the soil may play an important role, because they can bind selenium and reduce its bioavailability to plants. Microorganisms that can convert selenium from insoluble forms to soluble forms, and rainfall, which can flush out selenium from the soil, also have to be considered. [13] Protein-rich foods like meat, chicken, fish, eggs and nuts, especially Brazil nuts, contain high levels of selenium because selenium is able to replace sulfur in amino acids. In contrast the concentration of selenium in fruit is low, which can be explained by the low protein fraction. The concentration of selenium in milk and milk products is negatively correlated with the fat content. [14]

2.1.4 Health effects of selenium

Selenium is important for human health [15] and shows ambivalent behavior. [16] Several important metabolic pathways such as thyroid hormone metabolism, antioxidant defense and immune function depend on selenium. [15] The range between required and toxic dose is very narrow. [16] The recommended daily intake (RDI) for an adult is 55 µg/d and the tolerable upper intake level (UL) is 400 µg/d. [17] The major natural source of selenium is food, and hence selenium intake depends on soil selenium levels. [18]

2.1.4.1 Health benefits of selenium

Selenium is incorporated in selenoproteins as selenocysteine (Figure 1), the 21st amino acid. 25 selenoproteins have been identified in the human proteome, but many have functions that have not been completely investigated. [19, 20] Most of them are redox enzymes, such as glutathione peroxidase (GPx), type 1 5’iodothyronine deiodinase, and thioredoxin reductases (TrxR). These enzymes protect cellular components from oxidative damage, are necessary for proper thyroid function, and maintain the intracellular redox state.

Selenoprotein P is an extracellular protein, which may serve as a transport protein for selenium from the liver to peripheral tissues. [19]

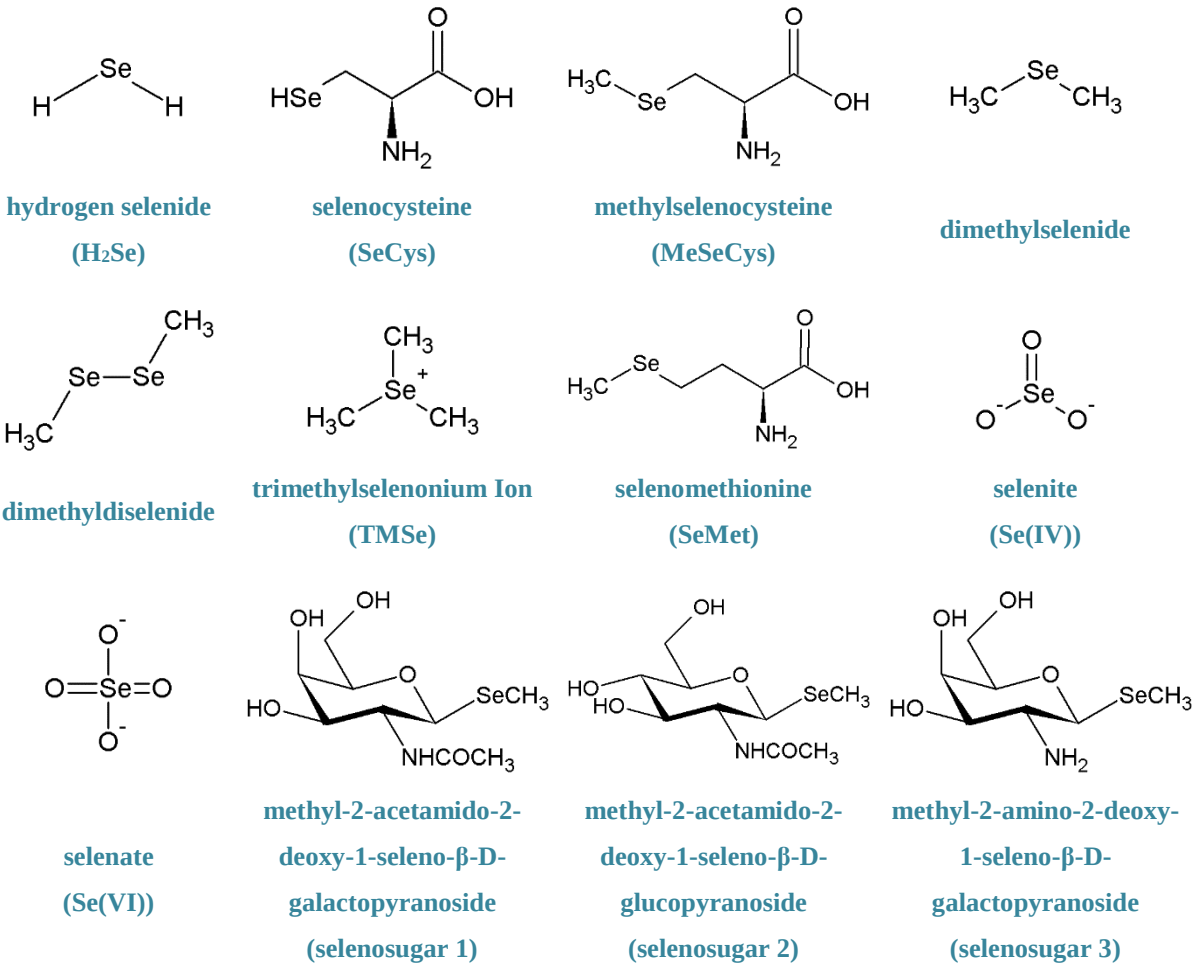

Figure 1: Structure of relevant selenium species

Numerous studies have indicated that insufficient selenium levels are directly or indirectly associated with several human diseases including cancer, diabetes, cardiovascular and immune system disorders. GPx and TrxR enzymes, which reduce oxidative stress, are very important for most of these associations, [21] because of the reduction of hydrogen peroxides, lipid and phospholipid hydroperoxides. Further, these enzymes reduce the propagation of free radicals and reactive oxygen species. [19, 20] Ca^{2+} signalling, spermatogenesis or brain function are also influenced by selenoproteins. [21] Selenium interacts with toxic metals and metalloids. For example, selenium is able to decrease the bioaccumulation of cadmium and arsenic [21] and counteracts mercury poisoning. [22]

Over the last 20 years, the ability of selenium to reduce the development of cancer has been investigated for a variety of cancer forms. Several studies showed that the ingestion of 200 µg Se/day reduces the risk of prostate, lung and bladder cancers, [18, 21] but other studies

found no significant effects of selenium supplementation. [23] A possible explanation for the different results could be that only specific selenium compounds have chemopreventive properties, since the studies used different selenium compounds for ingestion. [24] Furthermore, also human selenium status has to be considered because at very high selenium levels an additional selenium supplementation may even have opposite effects on human health. [25]

2.1.4.2 Toxicity of selenium

Before selenium’s essentiality was discovered, it was first known as a toxic element. [26] Chemical properties of selenium compounds, particularly their solubility, determine toxicity. The soluble selenium species selenomethionine, selenite and selenate are more toxic than insoluble selenium compounds. [27] Environmental toxicity of selenium in humans is much less common than selenium deficiency, but acute or chronic toxicity can appear by the intake of high-selenium containing dietary supplements. [28, 29] Selenosis is a specific pathology, which is caused by chronic oral intake of very high amounts of selenium. It is characterized by hair loss, deformation and loss of nails, abnormal functioning of the nervous system and other symptoms. [21]

2.1.5 Selenium metabolism

Human selenium metabolism is very complex and not completely understood. [14] It is known that the chemical form impacts on absorption, retention and, therefore, utilization. [30] Selenium compounds are absorbed from the human gastrointestinal tract at the lower end of the small intestine. [31] Soluble selenate and selenomethionine are most easily absorbed, [27] which is not the case for selenite. [32] Besides the chemical form of selenium, dietary factors also regulate bioavailability. For example, proteins and high levels of vitamin E can act as enhancers of uptake. Toxic elements, such arsenic or mercury, high levels of sulfur or vitamin C may be inhibitors for absorption. [31] Following absorption, the various selenium forms are metabolized into hydrogen selenide, which is further metabolized to selenoproteins. [14] Excretion occurs via three routes: urine, feces and breath, where selenium is exhaled in the form of volatile methylated metabolites. The major route for the elimination of excess selenium is the urinary pathway. When the dietary intake increases, urinary excretion also increases. [31]

2.2 Selenium in biological fluids with emphasis on human urine

Monitoring selenium body status is an important matter. [33] Several human body fluids and tissues, for example whole blood, blood plasma, blood serum, hair and toenails, have been discussed as indicators for nutritional selenium status. Human nails or hair show the exposure over the past 6 to 12 months, [14] but contamination, e.g. selenium-containing shampoo, must be avoided. [29] In contrast, blood and urine are biomarkers which show the selenium intake for no longer than several days (for urine) or several weeks (for blood). [14] Urine is frequently investigated because it is easily available. Furthermore, most trace elements, including selenium, are mainly excreted via the urine. [33] Several different selenium species are found in urine, but not all of them have been identified yet. First, it was reported that the trimethylselenonium ion ($TMSe^+$) is the major form of selenium in urine. Improved analytical methods have shown that TMSe ion is not generally a significant constituent of human urine. [34] Important constituents of human urine are selenosugars, [35] of which selenosugar **1** is usually the major identified urinary selenium species, whereas selenosugar **3** is minor, and selenosugar **2** has only been found after selenium supplementation. [34] Urinary selenium concentration in an unsupplemented, healthy person may usually be between 2 and 30 µg Se/L. [36] Therefore, techniques with low detection limits are required to measure selenium in urine.

2.2.1 Determination of total selenium

Direct determination of total selenium in biological fluids without sample preparation is only possible if the matrix does not affect the measurement and the measurement response of all contained selenium species is the same. High content of total dissolved solids (TDS) in body fluids can cause matrix effects and such samples often require sample preparation steps like digestion or dilution prior to the determination of total selenium. [33, 37] However, dilution and digestion are time-consuming and can lead to contamination or analyte loss. [33] Furthermore, some analytical techniques require the conversion of the different selenium species in a sample into one single chemical form [33] to overcome species dependent response. [38] This is particularly important for techniques based on hydride generation, [39] such as hydride generation/inductively coupled plasma mass spectrometry (HG/ICPMS), [40] hydride generation/atomic absorption spectrometry (HG/AAS) [41] or hydride generation/atomic fluorescence spectrometry (HG/AFS) [42] but also for other analytical techniques. For example, volatile species like dimethylselenide or dimethyldiselenide

(Figure 1) result in a higher response in ICPMS than non-volatile selenium species due to their more efficient transport from the spray chamber to the plasma. [37, 43]

Several measurement techniques have been applied for the determination of selenium in biological fluids. With electrothermal atomic absorption spectrometry (ETAAS) limits of detection (LOD) in the low µg/l range can be achieved for urine, [39, 44] which is in the range of the total selenium content in urine (2 – 30 µg Se/L). [36]. Additionally, some selenium compounds have a high volatility, which requires thermal stabilization in the ashing stage, and the various selenium species do not behave similar during pyrolysis. Also spectral interferences could occur because of several inorganic compounds like sulfate, phosphate and iron, which are also constituents of urine. [39, 44] In order to minimize analyte loss during the ashing stage, matrix modifiers, such as nickel, copper or palladium, are often used to make the analyte less volatile. [44, 45]

Hydride generation coupled to AAS [39, 41, 46, 47] or atomic fluorescence spectrometry (AFS) [48] is often used for the determination of low concentrations of selenium in complex sample matrices like urine. Detection limits in the sub µg Se/L range can be achieved. [46, 48] Selenite but also organic species, such as SeMet, $TMSe^+$ and selenosugars form volatile compounds after treatment with HCl and $NaBH_4$. [49] However, their efficiencies in forming volatile species differ. Therefore, it is necessary for total analysis to convert all selenium species into one single chemical form, usually selenite, prior to hydride generation. [39] Digestion and subsequent heating in HCl convert selenate to selenite, which is then transformed to hydrogen selenide, is usually applied. [41, 47, 50–54] Alternatively, selenium compounds can be directly transformed into selenite by mixtures of hydrobromic acid and bromine, *in situ* generated from potassium bromate. [55]

The conversion of selenium species to selenite is also required for the determination of selenium in body fluids by fluorimetry, which is based on the reaction of selenite with 2,3-diaminonaphthalene. [54, 56] However, this technique, although cost-effective, is of minor importance for urine analysis.

A robust technique with low detection limits for the determination of total selenium is ICPMS, [57] which is nowadays the method of choice for the analysis of selenium in body fluids. [58] The use of a collision/reaction cell is advantageous, because the most abundant selenium isotopes ^{80}Se (49.61 %) and ^{78}Se (23.78 %) are interfered by argon dimers ($^{40}Ar^{40}Ar^+$, $^{40}Ar^{38}Ar^+$). [59] When using H_2 as reaction gas, interferences of $^{79}Br^1H^+$ and $^{81}Br^1H^+$ occur on ^{80}Se and ^{82}Se when samples contain bromine, which is the case for urine and blood. [59]

2.2.2 Flow injection analysis

Flow injection analysis (FIA) is a simple and rapid sample introduction technique, which was first introduced in 1975 by Růžička and Hansen. [60] A large variety of detectors like mass spectrometers (MS), AAS, atomic emission spectrometers (AES), fluorimeters, electrochemical detectors, refractometers or spectrophotometers can be combined with FIA. [61] Liquid samples are injected into a continuous carrier flow, which transports the samples to the detector. Rapid, sequential analysis of a larger number of samples can be carried out with this technique. [62] Both the required time for sample preparation and sample volume can be reduced by using flow injection. The risk of sample contamination can be minimized, and the precision of measurements can be improved. [33] The basic components of a flow injection system are a pump, the injector, a mixing or reaction zone, and the detector (Figure 2). [62]

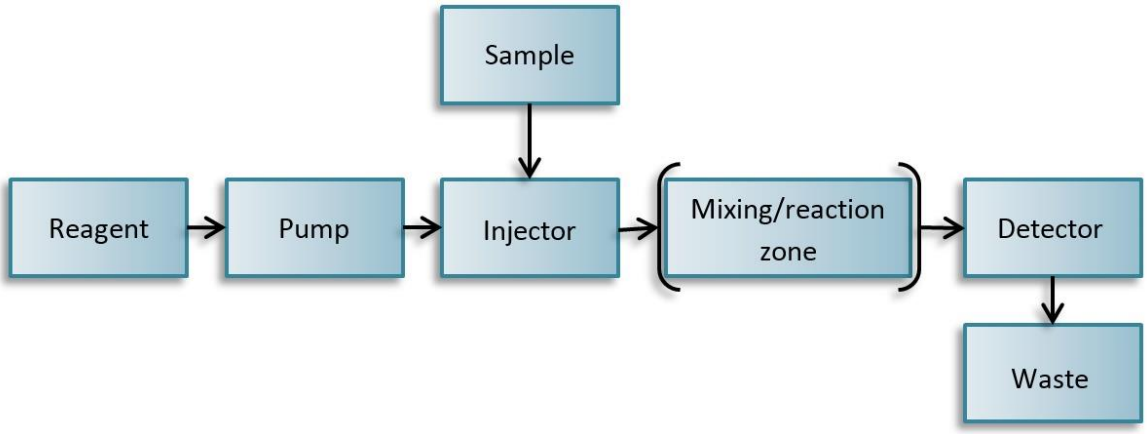

Figure 2: Design of a flow injection analyzer [62]

The *pump* moves the carrier solution through the flow injection system. The *injector*, which is used to place the sample in the carrier stream, is similar in nature and performance criteria to those used in HPLC. It is important that the sample solution is injected fast, so that the flow of the carrier solution is not disturbed. [62] After injection it can be necessary to transform the analyte into a species that can be measured by the *detector*. In this case the sample is mixed with a reagent in a reaction coil. For example, this is used in the hydride generation technique, in which the analyte is converted into a volatile species. The analyte or its derivate produces a signal at the *detector*, which is then used for quantification. [60]

At the time of injection the sample flow profile has a rectangular shape (Figure 3). [62] When the sample solution passes the tube, band broadening (dispersion) takes place. The shape is determined both by convection and diffusion. Dispersion by convection creates a parabolic shaped front. Laminar flow, in which the center of the fluid moves more rapid than the liquid

near to the walls, is the source of convection. Dispersion by diffusion occurs due to the concentration gradient between the sample and the carrier stream. [62]

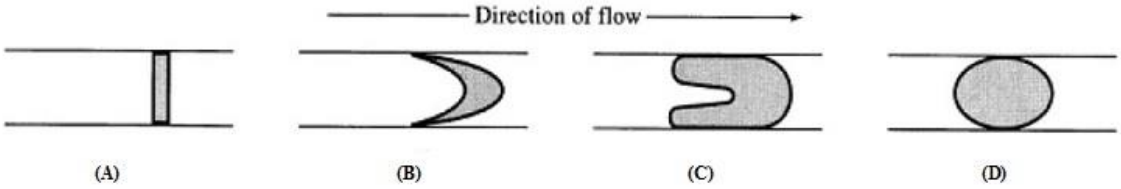

Figure 3: Impact of convection and diffusion on the concentrations profiles of the analytes at the detector: (A) no dispersion at injection, (B) dispersion by convection, (C) dispersion by convection and radial diffusion, (D) diffusion dominates dispersion [63]

Dispersion is the ratio between the initial analyte concentration and the concentration, which appears as a peak at the detector. [64] It is determined by the injection volume of the sample, the flow rate, the diameter of the tubing used and, if applicable, the length of the reaction coil. Hence the time between injection and detection is reproducible, which allows quantification. [60, 65] Dispersion is equal to 1 at large sample volumes, where no significant mixing of the sample with the carrier stream occurs, and no sample dilution takes place. If a derivate of the analyte should be generated by a reagent, a dispersion greater than one is required. For example, a dispersion of 2 is needed, when sample and reagent are to be mixed in a ratio of 1:1. Longer flow paths result in higher dispersion due to increased convection and diffusion, whereby the signal gets broader. [66]

The FIA curve (Figure 4) has the form of a peak, in which the height H, width W and area A are related to the concentration of the analyte. [62, 65] The time, in which a sample moves from the injector to the detector, is called travel time t_a. In contrast, the time, which is required to obtain the peak maximum is called residence time T. If convection is the primary source of dispersion, the difference (t’) between the residence time and travel time approaches zero. The difference between t_a and T increases as diffusion becomes more influential. The baseline-to-baseline time Δt is the time required for the sample to pass through the flow cell of the detector and for the signal to return to the baseline. The return time T’ is the elapsed time between signal maximum and its return to the baseline. It determines the frequency, with which samples may be injected. [62]

Evaluation of signals can be done by peak height H or peak area A, after calibration with suitable reference samples. Both are directly related to the analyte concentration. Another possibility is the peak width W, which is proportional to the logarithm of the concentration and

has a wide dynamic range. Compared to peak height or area measurement its precision is lower. [65]

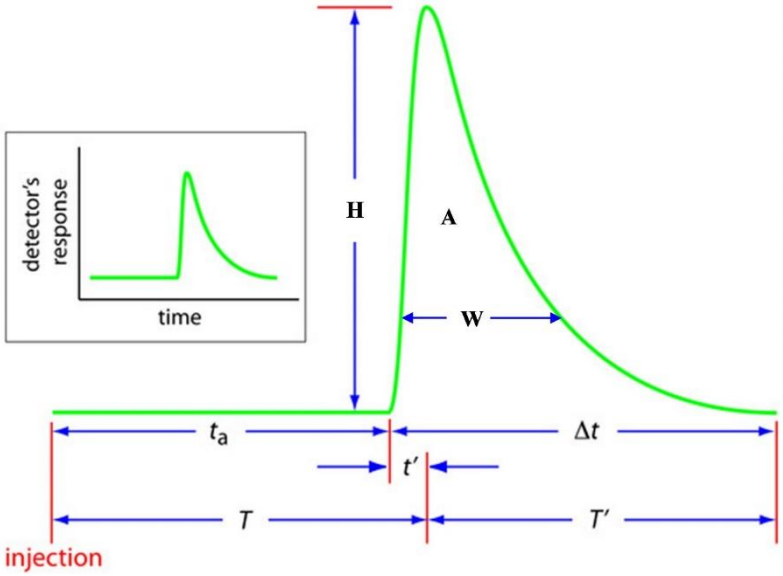

Figure 4: Typical FIA curve for flow injection analysis [62]

Several publications report the determination of total selenium in biological fluids by flow injection with HG/AAS, HG/ICPMS or ICPMS detection, whereby sample preparation by digestion or dilution was most frequently applied. Detection based on HG requires conversion of all selenium species into selenite. Therefore, digestion followed by heating with HCl to reduce selenate, which might be formed during digestion and is not hydride active, to selenite is often applied. [41, 47, 52, 53, 67] Alternatively digestion with a mixture of bromate and hydrobromic acid immediately transforming the selenium species into selenite was suggested (see also *Chapter 2.2.1*). [39, 47]

Among techniques based on flow injection, FI/HG/AAS was employed first for the determination of total selenium in body fluids (Table 1). In two publications a special instrumental set-up using a graphite tube, in which the formed hydrogen selenide was trapped, instead of a heated quartz tube as atomizer was employed. [39, 47] Digests of biological fluids (plasma, serum, or urine) containing all selenium as selenite were usually directly injected into the HCl solution used to generate hydrogen selenide by subsequent mixing with sodium borohydride (Table 1). Besides time-consuming sample preparation procedures necessary for techniques based on hydride generation, another disadvantage is the rather high injection volume used with these methods compared to flow injection performed with HPLC systems. Accuracy of the reported FI/HG/AAS was demonstrated with reference materials, [39, 41, 47, 53] comparison to results obtained by alternative techniques, [47, 52] as

Table 1: Overview of the literature for the determination of total selenium content in biological fluids by flow injection with various detectors

Method	Sample matrix	Sample preparation	Calibration	Carrier solution	Internal standard	Injection volume [µl]	LOD [µg Se/l]		Reference
							in solution	in sample	
FI/HG/AAS	blood plasma and serum	digestion, reduction by heating with HCl	external	1 M HCl	-	330	1.2	15	[52]
FI/HG/AAS	serum	digestion, reduction by heating with HCl	external	1 M HCl	-	500	0.3	6	[53]
FI/HG/AAS	urine	digestion	external	10 % HCl	-	500 / 1000	1.2	6	[39]
FI/HG/AAS	urine	digestion, reduction by heating with HCl	external	1 M HCl	-	500	0.3	3	[41]
FI/HG/AAS	urine	digestion, reduction by heating with HCl	external	not reported	-	not reported	0.6	15	[47]
FI/HG/ICPMS	serum	dilution, reduction by heating with HCl	external	0.6 M HCl	-	100	0.035	3.5	[67]
FI/ICPMS	urine	filtration, dilution	not reported	not reported	Ge	100	6.5	-	[68]
FI/ICPMS	urine	dilution	standard addition	0.65 % (m/v) HNO_3	Rh	not reported	0.45	-	[33]
FI/ICPMS	urine	-	standard addition	1 % HNO_3	Rh	821	not reported	not reported	[69]
FI/ICPMS	blood serum and plasma	filtration, dilution	standard addition	phosphate buffer (pH 6.8)	-	50	not reported	not reported	[70]
FI/ICPMS	serum, cerebrospinal fluid	dilution	external	Milli-Q	Rh	25	0.026	1.3, 0.026	[71]

well as spiking recoveries. [39, 41, 52] Although the typical precision of about 5 % RSD [41, 52, 53] is satisfactory, the obtained LODs (3 – 15 µg Se/l) are too high for the determination of total selenium in normal background urine (Table 1).

FI/HG followed by the detection of serum selenium with ICPMS was reported by Quijano et al. [67] Diluted serum samples were mixed with concentrated hydrochloric acid and boiled to convert selenate to selenite. When a serum sample was spiked with different amounts of selenate, recoveries were quantitative. However, the native serum before spiking contained only 35 µg Se/L which is at the lower limit for total selenium in serum. [14] This might indicate that not all of the contained selenium was detected because selenium species other than selenate might not have been quantitatively converted into selenite without a digestion step prior to reduction by HCl.

Centineo et al. were unable to accurately determine the selenium concentration in a human urine certified reference material by FI/HG/ICPMS after filtration and dilution as the only sample preparation steps, [68] and, therefore, used flow injection directly coupled to ICP/time of flight (TOF) MS to achieve accurate results. However, the achieved LOD of the FI/ICPTOFMS method was still too high to make it suitable for the determination of total selenium in background urine. Lower detection limits were achieved by coupling flow injection to a conventional ICPMS system. [33] On-line dilution was used to decrease matrix effects. Nevertheless, the time-consuming approach of standard addition calibration was necessary to obtain accurate results. There is only one report where total selenium in biological fluids was determined by FI/ICPMS without sample preparation. [69] For quantification of total selenium in urine, online standard addition was carried out by injecting a small volume of standard as a spike into the sample/carrier stream. With this set-up, and the use of an internal standard, matrix effects could be accounted for without time-consuming calibration procedures. The method was applied to a certified urine reference material, but no other performance characteristics of the method like an LOD were reported. [69]

Although the use of injection volumes below 100 µL with an HPLC system instead of a conventional flow injection system for FI/ICPMS should result in less matrix effects, standard addition was still necessary for the quantification of total selenium in blood serum and plasma. [70] The latest FI/ICPMS study [71] investigated cerebrospinal fluid (CSF) and serum samples but also one urine control material with external calibration. Data evaluation using ^{80}Se was possible because of the use of methane as reaction gas precluding interferences of $^{1}H^{79}Br$ known to hamper quantitation on ^{80}Se, when hydrogen is used as reaction gas. [59] Therefore, an LOD of 0.026 µg Se/L was achieved with this method. However, when undiluted

cerebrospinal fluid was analyzed, strong signal suppression was observed for rhodium, which was used as internal standard indicating severe matrix effects. To minimize these effects, serum samples, which contain about 10 to 50-fold higher total selenium concentrations than cerebrospinal fluid, were diluted with water before measurements. [71]

Generally, accurate results were obtained with flow injection coupled to ICPMS detection as demonstrated by the analysis of suitable reference materials, [33, 69–71] comparison with results obtained by alternative techniques, [70, 71] as well as spiking recovery experiments. [33, 68, 71] The RSDs were as satisfactory as those obtained by AAS detection. Injection volumes were reduced to a minimum of 25 µL with this technique, which is particularly important for body fluids, of which only low amounts are available, and LODs of the recent methods were suitable for the analysis of background urine. However, time-consuming sample preparation steps like dilution [33, 68, 70, 71] or manual addition of an internal standard, [68, 71] as well as standard addition calibration [33, 69, 70] were still necessary, which is not ideal when large numbers of samples have to be analyzed.

2.3 Aim of the work

Total selenium in urine is often used for assessment of recent selenium intake. A simple, accurate, and precise high-throughput method is desirable for the analysis of a large number of samples to avoid time-consuming sample preparation, such as digestion or dilution. The aim of this work was to develop a method for measuring total selenium in urine by FI/ICPMS with external calibration without prior dilution or digestion. The method should be validated with respect to accuracy and precision in the urinary matrix as well as its ability to reliably determine total selenium in urine samples that had been stored for longer periods of time before analysis.

3 Experimental

3.1 Materials and methods

3.1.1 Chemicals and reagents

All solutions were prepared with Milli-Q water (18.2 MΩ cm, Milli-Q® Academic water purification system, Millipore GmbH, Vienna, Austria). Chemicals and reagents used are summerized in Table 2. Stock standard solutions of the selenium compounds were prepared in water at the following concentrations: selenomethionine 90 mg Se/L, selenite 1000 mg Se/L, selenate 1000 mg Se/L, TMSe 1000 mg Se/L, selenosugar **1** 65 mg Se/L and methylselenocysteine 100 mg Se/L. Working solutions of standards were prepared daily by step-wise dilution of the stock standard solutions with water. Chemicals were used without further purification except for nitric acid, which was further purified in a quartz sub-boiling distillation unit.

3.1.2 Certified reference materials

The accuracy of the calibration for total measurements by ICPMS after microwave-assisted acid digestion was checked by analysis of the reference water SRM 1640a (Trace Elements in Water, National Institute of Standards and Technology (NIST), Gaithersburg, USA; certified: 19.97 ± 0.16 µg Se/L, found: 19.90 ± 0.26 µg Se/L, n = 6). In addition the reference material ClinCheck® Urine Controls (Lyophilized, for Trace Elements, Level I, Recipe®, Munich, Germany; certified: 79.0 ± 15.8 µg Se/L, found: 80.7 µg Se/L and 83.3 µg Se/L, n = 2), the certified reference material NIES No. 18 Human Urine, (National Institute For Environmental Studies, Tsukuba, Japan; certified: 59 ± 5 µg Se/L, found: 62 ± 2 µg Se/L, n = 4) and urine from a proficiency test (53. Ringversuch 2014 für toxikologische Aspekte in biologischem Material; Institut und Poliklinik für Arbeits-, Sozial- und Umweltmedizin der Universität Erlangen-Nürnberg; target value: 84.3 ± 15.9 µg Se/L, found: 81.9 ± 1.6 µg Se/L, n = 5) were used for quality control of the conventional method.

The accuracy of the developed FI/ICPMS method was checked with the certified reference material NIES No. 18 Human Urine (certified: 59 ± 5 µg Se/L, found: 61.4 ± 0.4 µg Se/L, n = 3).

Table 2: Chemicals and reagents

Chemical / Reagent	Concentration	Quality	Manufacturer
Germanium stock solution	1000 ± 3 mg/L in 2 % HNO_3	Peak Performance	CPI International, Santa Rosa, USA
Selenium stock solution	1000 ± 0.2 mg/L in 2 % HNO_3	Roti®Star, Single-Element ICP-Standard-Solution	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Multi-element standard solution	100 mg Se/L	Certipur®, ICP multi-element standard solution VI for ICP-MS, 30 elements in dilute nitric acid	Merck Millipore KGaA, Darmstadt, Germany
Selenomethionine	-	≥ 99,0 % (TLC)	Fluka, Buchs, Switzerland
Sodium Selenite	-	> 99 %	Merck, Darmstadt, Germany
Sodium Selenate	-	> 99 %	Fluka, Buchs, Switzerland
Methylselenocysteine	-	≥ 99,0 % (TLC)	Sigma-Aldrich Chemie GmbH, Munich, Germany
Trimethylselenonium iodide	-	-	Synthesized in-house according to [72]
Selenosugar 1	-	-	Synthesized in-house according to [73]
Nitric acid	≥ 65 %	Rotipuran®, p.a., ISO	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Formic acid	≥ 98 %	Rotipuran®, p.a., ACS	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Sulfuric acid	≥ 95 %	Rotipuran®, p.a., ISO	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Methanol	99.9+ %	HPLC gradient grade	Chem-Lab N.V., Zedelgem, Belgium
	-	HPLC gradient grade	VWR Prolabo Chemicals, Fonenay-seous-Bois, France
Ammonium formate	≥ 95 %	purum	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Malonic acid	≥ 98 % (T)	purum	Fluka Chemie GmbH, Buchs Switzerland
	99 %	-	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Aqueous ammonia	≥ 25 %	Rotipuran®, p.a.	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

3.1.3 Consumables

Consumables used in this work are illustrated in Table 3.

Table 3: Consumables

Consumable	Manufacturer
Cellstar® Tubes, 50 mL, polypropylene (PP), graduated, conical bottom, blue screw cap, sterile	Greiner Bio-One GmbH, Frickenhausen, Germany
Cellstar® Tubes, 15 mL, PP, graduated, conical bottom, blue screw cap, sterile	Greiner Bio-One GmbH, Frickenhausen, Germany
Polystyrol (PS) Tubes, 12 mL	BRAND, Wertheim, Germany
Micro tubes, 1.5 mL, PP	Plastibrand®, Wertheim, Germany
Pipette tip blue	Greiner Bio-One GmbH, Frickenhausen, Germany
Pipette tip yellow	Greiner Bio-One GmbH, Frickenhausen, Germany
Parafilm, PM 996, Bemis Flexible Packaging	Bemis Company Inc, Neenah, USA
TERUMO®, Syringe without Needle, 6 % LUER, 2 mL	Terumo Europe N.V., Leuven, Belgium
Pro Fill 25 mm HPLC syringe filter, Nylon (PA), porosity 0.2 µm	Markus Bruckner Analysentechnik, Linz, Austria
HPLC micro vials, Vial crimp/snap, PP, 1 mL	Agilent, Waldbronn, Germany
HPLC micro vials, Vial crimp/snap, PP, 250 µL	Agilent, Waldbronn, Germany
HPLC caps, Al CRIMP CAP PTFE/Rubber TF2 SEPT, 11 mm	Agilent, Waldbronn, Germany
Wide Mouth Snap-Seal Sample/Specimen Container with Snap Cap, PP, graduated and non-sterile, 300 mL	Corning Inc., New York, USA

3.1.4 Instrumentation

For conventional total measurements and FI/ICPMS an Agilent 7500ce ICPMS system (Agilent Technologies, Waldbronn, Germany) equipped with an Integrated Sample Introduction System (ISIS), an autosampler (Agilent ASX 500 Series), a cyclonic spray chamber (Elemental Scientific Inc, Omaha, USA), and a Burgener Ari Mist HP Nebulizer (Burgener Research International, Berkshire, UK) was used. Data evaluation was carried out with ICPMS Masshunter software (G72008, B.01.01).

An Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of an eluent degasser (G 1379 A), a binary pump (G 1312 A), a thermostated autosampler (G 1329 A), and a thermostated column oven (G 1316 A) was used for speciation analysis and FI/ICPMS. Further instrumentation used in this work is summerized in Table 4.

Table 4: Instrumentation

Instrumentation	Type	Manufacturer
Balances	S-4001, Denver Instrument	Sartorius, New York, USA
	SI-234, Denver Instrument	Sartorius, New York, USA
Micropipettes	ACURA 826, 10 – 100 µL, autoclavable, XS	Socorex, Ecublens, Switzerland
	ACURA 826, 100 – 1000 µL, autoclavable, XS	Socorex, Ecublens, Switzerland
Sub-boiling Unit	duoPUR MLS	EMLS, Leutkirch, Germany
pH meter	pH 1000 L	pHenomenal®, VWR®
	Electrode: 6.0253.100 Aquatrode Plus	Metrohm AG, Herisau, Switzerland
Digestion system	UltraCLAVE 4, High Performance Microwave Reactor, Milestone	MLS GmbH, Leutkirch, Germany
with:	Quartz Tubes, 12 mL, with Teflon cap, Teflon rack	
Dispenser	Fortuna® OPTIFIX® Safety 10	Poulten & Graf GmbH, Wertheim, Germany
Refractometer	Leica TS 400®, Serum Protein 6.54	Leica Microsystems Inc., New York, USA
Passive splitter	QuickSplit™ Adjustable Flow Splitter	Analytical Scientific Instruments, USA
HPLC Columns	Atlantis® dC18, 5 µm, 4.6 x 150 mm	Waters, Dublin, Ireland
	PRP-X100, 5 µm, 4.6 x 150 mm	Hamilton Company, Nevada, USA

3.2 Sample collection

Human urine (18 samples) was collected from 11 volunteers into 300 mL plastic bottles, aliquoted into 15 mL tubes or 1.5 mL micro tubes and stored at -20 °C. On the day of measurement, the aliquots were thawed and prepared for measurement. In order to investigate the applicability of the method, different urine samples were collected to cover a range of selenium concentrations and specific gravities. The specific gravity of the urine samples was measured with a refractometer.

3.3 Determination of total selenium by ICPMS after microwave-assisted acid digestion (conventional method)

Total selenium concentrations in the urine samples were determined by ICPMS after mineralization of the samples by microwave-assisted acid digestion (hereafter named “conventional method”).

3.3.1 Sample preparation

500 µL of the urine samples (unfiltered or filtered) or of the certified reference material NIES CRM No. 18, or 200 µL of the reference material ClinChek Urine and of the reference urine from a proficiency test were transferred into quartz digestion vessels of the UltraClave 4. Then 2 mL Milli-Q water and 2 mL nitric acid were added. The quartz tubes were placed in a Teflon® rack and covered with Teflon® caps. An absorber bath consisting of 300 g of Milli-Q water and 5 g of sulfuric acid was used. The digestion system was closed, loaded with argon to 40 bar and the microwave program was initiated. After digestion the digests were transferred to 15 mL tubes and diluted to a final volume of 10 mL with Milli-Q water. Then, 100 µL of an internal standard (2.5 mg/L Ge in 2 % HNO_3) were added to the digested solutions.

3.3.2 Determination of total selenium by ICPMS

Determination of total selenium concentrations in the urine samples was carried out by ICPMS. An octopole reaction cell with H_2 as reaction gas (flow rate: 3.5 mL/min) was used to reduce polyatomic interferences on ^{78}Se and ^{80}Se resulting from the argon dimers ($^{40}Ar^{40}Ar^+$ and $^{40}Ar^{38}Ar^+$). A mixture of 1 % CO_2 in Ar (12 % of the carrier gas) was used as optional gas to enhance the selenium signal. The selenium isotopes ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se were measured; quantification was performed on *m/z* 78 because of possible interferences of HBr with ^{80}Se and ^{82}Se when H_2 is used as reaction gas.

Before measurement, the instrument was tuned to maximum sensitivity and low oxide ratios with a solution consisting of 1 µg/L of Li, Y, Tl, Ce, Co, Fe, Se, As and Cr in 2 % HNO_3 (*Figure A. 1*, Appendix). External calibration was carried out with standard solutions of selenium (0.05 – 5 µg Se/L) prepared from the Single-Element ICP-Standard-Solution (hereafter named “single-element-standard”) in 20 % HNO_3. The instrument drift was generally < 5 %. Quantification was done using ^{72}Ge as internal standard.

3.4 Determination of total selenium by flow injection / ICPMS

FIA was carried out by coupling the HPLC system with a PEEK (polyetheretherketone) capillary tubing (0.125 cm i.d.) to the nebulizer of the ICPMS (for typical ICPMS settings see *Figure A. 1*, Appendix). The signals on *m/z* 78 and 80 were monitored, whereby quantification was performed on *m/z* 78. External calibration was carried out with standard solutions containing 1 – 25 µg Se/L (in some cases up to 50 or 100 µg Se/L depending on the selenium content expected in the samples) prepared from the single-element-standard. Standard addition was sometimes performed, whereby aliquots of the filtered urine samples were spiked with different concentrations of the single-element-standard (50 µL of single-element-standard was added to 2 mL urine sample). Urine samples were filtered through 0.20 µm Nylon filters prior to analysis. Unless otherwise stated, each standard and urine sample was injected three times and mean and standard deviation were calculated. As drift control and accuracy control for the calibration a TMSe standard (20 µg Se/L) was also analyzed. The drift was always < 5 %.

3.5 Determination of selenium species in two commercially available selenium standards by HPLC/ICPMS

To investigate the selenium species present in the commercially available ICP-Multi-Element-Standard-Solution (hereafter named “multi-element-standard”) and single-element-standard, anion-exchange HPLC/ICPMS was employed using a PRP-X100 column (5 µm, 4.6 x 150 mm, Hamilton Company, Nevada, USA) and 20 mM malonate pH 9.5 as mobile phase. The flow rate was 1.0 mL/min, the column temperature was 30 °C, and the injection volume was 20 µL. A standard solution containing selenite and selenate (20 µg Se/L each) was used for identification of the species by retention time matching.

3.6 Optimization of FI/ICPMS

3.6.1 Carrier solution

Different carrier solutions were investigated. Ammonium formate (20 mM with and without 3 % MeOH, pH 3.0 adjusted with formic acid), malonate (20 mM with and without 3 % MeOH, pH 3.0, 6.0 or 9.5 adjusted with aqueous ammonia) and HNO_3 (0.005 M in 10 % MeOH) were checked. The selenium species selenite, selenate, selenosugar **1**, TMSe, SeMet, MeSeCys and two commercial selenium standards, the multi-element-standard and the single-element-standard, at concentrations of 20 µg Se/L each were measured. Urine samples were quantified using external calibration or standard addition. All selenium standards were injected twice and Milli-Q water was injected between two different selenium species to investigate carry-over. Measurements performed using flow splitting were conducted with the set-up usually employed for total measurement (see *Chapter 3.6.2*). Measurement parameters are summarized in Table 5 (a) and (b).

3.6.2 Flow splitting

Flow splitting was employed to reduce matrix effects. In this set-up only a part of the injected sample is directed to the ICPMS. Flow splitting was carried out with two different set-ups: (i) a set-up, which was also used for total measurements to allow faster filling of the tubing from the autosampler to the ICPMS, employing a T-piece in front of the nebulizer pump (total-set-up), and, (ii) a passive splitter (Figure 5). Flow splitting in the total-set-up was performed by coupling the HPLC system with PEEK capillary tubing (0.125 cm i.d.) to a T-piece. One exit of the T-piece was further directed to the waste (65 % of flow) and the other exit to the nebulizer pump of the ICPMS (35 %).

In the other set-up, the HPLC system was coupled with a PEEK capillary tubing (0.090 cm i.d.) to a passive splitter. Two more PEEK capillary tubings were connected to the passive splitter: one was coupled to the nebulizer of the ICPMS and the second was linked to the waste. The advantage of the second set-up was the lower dead volume. Different split ratios (10 : 90, 15 : 85, 20 : 80, 25 : 75 and 35 : 65, Table 5 (c)) were tested at a constant injection volume (20 µL) and flow rate (1.0 mL/min) using the passive splitter and 20 mM malonate 3 % MeOH pH 6.0 as carrier solution. Results were compared with those obtained without flow splitting. The same samples as detailed in *Chapter 3.6.1* were measured.

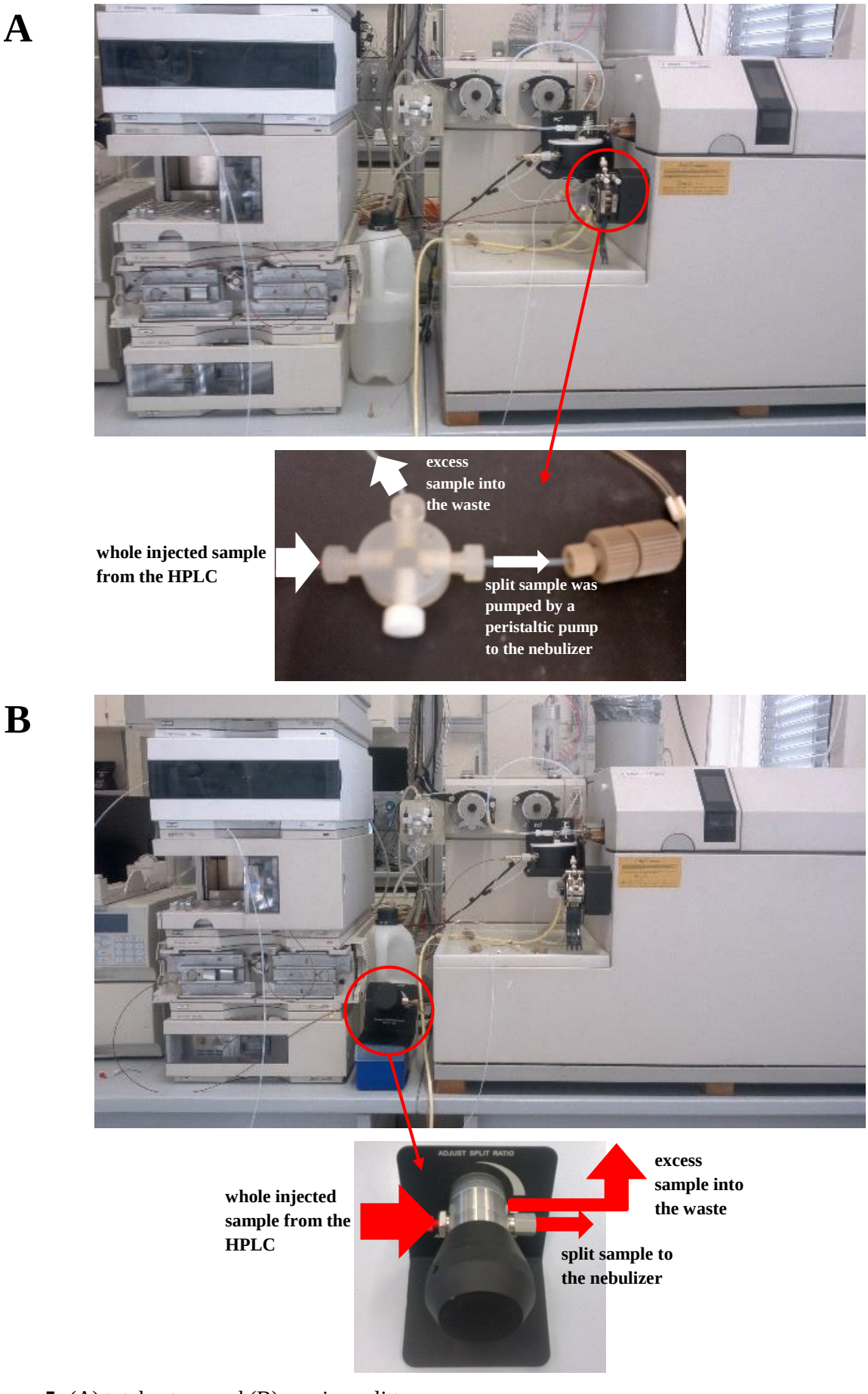

Figure 5: (A) total-set-up and (B) passive splitter

Table 5: Measurement parameters

Injection volume [µL]	Flow rate [mL/min]	Integration time [s/point]	Split ratio [% directed to ICPMS : % directed to the waste]
(a) without flow splitting			
2	0.4	0.3	-
(b) flow splitting using the total-set-up			
20	1.0	0.3	35 : 65
(c) flow splitting using the passive splitter			
20	1.0	0.3	10 : 90 – 35 : 65

3.6.3 Flow rate

Different flow rates (1.0, 1.5, and 2.0 mL/min) were studied with 20 mM malonate 3 % MeOH pH 6.0 as carrier solution at an injection volume of 20 µL and an integration time of 0.3 s/point. The passive splitter was used with a split ratio of 15 : 85. Urine samples and standard solutions (see *Chapter 3.6.1*) were used for evaluation. Total selenium in the urine samples was quantified using external calibration.

3.6.4 Integration time

The influence of different integration times on the peak shape of the FIA curve was investigated with the single-element-standard (1 µg Se/L) at different integration times (0.1 s/point, 0.2 s/point and 0.3 s/point). A constant injection volume (20 µL), a flow rate of 1.5 mL/min and a split ratio of 15 : 85 (passive splitter) were chosen using 20 mM malonate 3 % MeOH pH 6.0 as carrier solution.

3.6.5 Injection volume

To investigate the influence on signal suppression, injection volumes in the range of 1 – 20 µL were tested with 20 mM malonate 3 % MeOH pH 6.0 as carrier solution at a flow rate of 1.5 mL/min, an integration time of 0.1 s/point and a split ratio of 15 : 85 (passive splitter). Draw speed and eject speed were reduced at lower injection volumes to avoid decrease of the injection volume precision. Total selenium in urine samples was quantified using external calibration.

3.6.6 Internal standard

In order to compensate for matrix effects germanium was used as internal standard. Germanium was added to the carrier solution (final concentration 50 µg Ge/L) or 1 µL of 50 µg Ge/L in carrier solution was spiked to the samples with an injection program performed by the HPLC autosampler. 20 mM malonate 3 % MeOH pH 6.0 was used as carrier solution and different injection volumes (2 µL, 5 µL, 10 µL and 15 µL) were investigated. External calibration was used for quantifying total selenium in urine samples. FI/ICPMS parameters were as follows: flow rate, 1.5 mL/min; split ratio, 15 : 85; integration time, 0.1 s/point.

Results with internal standard correction were compared to those obtained without correction. Internal standard correction for germanium in the carrier solution was performed by dividing the signal on *m/z* 78 by the signal on *m/z* 72 for every time point in the FIA curve (Equation 1). The corrected signal was then integrated with the in-house-programmed software WINFAAS.

$$signal_{corrected} = \frac{signal_{m/z\,78}}{signal_{m/z\,72}} \qquad \text{Equation 1}$$

Quantification in the case of the method using the injection program was performed with the following calibration curve considering the internal standard.

$$\frac{Peak\ Area_{m/z\,78}}{Peak\ Area_{m/z\,72}} = k * \frac{selenium\ concentration}{germanium\ concentration} + d \qquad \text{Equation 2}$$

with: *k: slope of the calibration curve*

d: intercept of the calibration curve

3.7 Analytical performance

Method performance parameters like linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) of the FI/ICPMS method were determined using the following optimized settings: injection volume: 5 µL; carrier solution: 20 mM malonate 3 % MeOH pH 6.0 containing 50 µg Ge/L as internal standard; flow rate: 1.5 mL/min; integration time: 0.1 s/point; split ratio: 15 : 85 (passive splitter). External calibration was performed with single-element standard solutions containing 1 – 100 µg Se/L.

The accuracy of the FI/ICPMS method was checked in three different ways:

(i) NIES No. 18 Human Urine, a certified reference material, was measured after filtration and the results were compared to the reported certified value.

(ii) 10 different urine samples were measured with the conventional method (see *Chapter 3.3*) and the optimized FI/ICPMS method.

(iii) Spiking experiments were carried out by spiking 2 mL of urine with 0.05 mL of a standard solution containing 250 µg Se/L or 740 µg Se/L.

Intra-day precision, expressed as RSD (%), was determined by injecting 3 different urine samples ten times each on the same day, whereas both intra-day and inter-day precision were evaluated with 10 different urine samples (three replicate injections) at three or four different days. Intra-day and inter-day precision were calculated using the ANOVA function in MS Excel.

LOD and LOQ were determined in the urinary matrix by using urine samples with very low total selenium concentrations (1 – 2 µg Se/L) injected ten times. To calculate the LOD, the standard deviation of the peak area of the ten injections was multiplied by 3 and transformed into a concentration via the calibration curve. The LOQ was determined in the same way by using the 10-fold standard deviation of the peak area.

3.8 Stability of the urine samples

The stability of six urine samples with different specific gravities and selenium concentrations at 4 °C was tested to check how long samples could be stored before the optimized method begins to produce wrong results. One of the urine samples was spiked with selenosugar **1** to a final concentration of 30 µg Se/L. Furthermore, the stability of the calibration standards (2 – 50 µg Se/L) upon storage at 4 °C was tested. Determination of total selenium by the optimized method (injection volume: 5 µL; carrier solution: 20 mM malonate 3 % MeOH pH 6.0 containing 50 µg Ge/L as internal standard; flow rate: 1.5 mL/min; integration time: 0.1 s/point; split ratio: 15 : 85 using the passive splitter) was performed at the beginning of the experiment and periodically after 1, 2, 3, 4, 7, 10 and 14 days. Selenium species contained in the urine samples before and after storage for 4 and 14 days were monitored by reversed-phase HPLC/ICPMS analysis (column: Atlantis® dC18, 5 µm, 4.6 x 150 mm; mobile phase: 20 mM ammonium formate 3 % MeOH pH 3.0; injection volume: 20 µL; flow rate: 1.0 mL/min; temperature of the column oven: 30 °C). External calibration was carried out with standard solutions of TMSe and selenosugars **1** and **3** (0.5 – 30 µg Se/L each).

4 Results and discussion

The aim of this work was to develop a method for determining total urinary selenium concentrations by FI/ICPMS without time-consuming sample preparation procedures like dilution or digestion. Quantification should be carried out by external calibration. The development of the method was carried out by optimization of a variety of parameters, like carrier solution, flow splitting, integration time, flow rate, injection volume, and the use of an internal standard.

4.1 Determination of total selenium in urine by microwave-assisted acid digestion and subsequent ICPMS measurement (“conventional method”)

The total selenium concentrations in the urine samples used for FI/ICPMS measurements were determined with the conventional method, i. e. microwave-assisted digestion and measurement by ICPMS using ^{72}Ge as internal standard. Furthermore, the specific gravity of each urine sample was measured, whereby no differences between filtered and unfiltered urine samples were observed. As shown in Table 6 urinary selenium concentrations, ranged from 1.2 – 29.8 µg Se/L for 11 volunteers, which matched the reported urinary selenium concentration in non-supplemented, healthy persons (2 to 30 µg Se/L). [36] Urine collected in the morning typically contained a higher selenium concentration and had a higher specific gravity than urine from the same volunteer collected in the evening. Therefore, higher specific gravities could be related to higher selenium concentrations in the urine samples. The differences between unfiltered and filtered urine samples were minimal (Table 6). The results obtained from this conventional method were subsequently used to test the validity of the FI/ICPMS method (see *Chapter 4.3*).

4.2 Selenium species in two commercially available selenium standards by HPLC/ICPMS

To minimize potential sources of error in quantification, standards with guaranteed selenium concentrations are advantageous for preparation of calibration curves. Two commercial standards were available, a multi-element-standard containing 30 different elements and a single-element-standard only containing selenium. To investigate the selenium species in these standards anion-exchange HPLC/ICPMS was employed. The chromatograms of the two

selenium standards (20 µg Se/L each) were compared with a selenite and a selenate standard (20 µg Se/L each). Both standards contained only inorganic selenium. The single-element-standard was mainly in the form of selenite with approximately 10 % being selenate. In contrast, the multi-element-standard contained only selenite (Figure 6). The single-element-standard was chosen for quantification in all further FI/ICPMS, because it is cheaper than the multi-element-standard.

Table 6: Total urinary selenium concentrations (mean ± standard deviation of three digests) and specific gravities of human urine samples used in this work

Sample	Concentration [µg Se/L]		Recovery after filtration [%]	Specific gravity	Time of collection	Volunteer
	unfiltered	filtered				
Urine 1	20.4 ± 0.1	19.0 ± 0.3	93	1.016	Morning	1
Urine 2	9.2 ± 0.1	8.9 ± 0.1	98	1.014	Evening	2
Urine 3	23.0 ± 0.5	21.0 ± 0.8	91	1.024	Morning	2
Urine 4	-	7.6 ± 0.1	-	1.011	Evening	3
Urine 5	-	10.7 ± 0.1	-	1.012	Morning	3
Urine 6	-	6.5 ± 0.1	-	1.011	Evening	4
Urine 7	-	20.0 ± 0.1	-	1.020	Morning	4
Urine 8	-	12.9 ± 0.1	-	1.012	Evening	5
Urine 9	-	18.6 ± 0.2	-	1.015	Morning	5
Urine 10	-	9.9 ± 0.3	-	1.013	Evening	6
Urine 11	-	7.9 ± 0.1	-	1.011	Morning	6
Urine 12	-	7.8 ± 0.2	-	1.007	Evening	7
Urine 13	-	10.3 ± 0.2	-	1.010	Morning	7
Urine 14	-	1.2 ± 0.1	-	1.002	Evening	8
Urine 15	-	2.5 ± 0.1	-	1.003	Evening	9
Urine 16	-	1.9 ± 0.2	-	1.002	Evening	10
Urine 17	11.2 ± 0.1	10.6 ± 0.3	95	1.009	Evening	11
Urine 18	30.3 ± 0.1	29.8 ± 0.1	98	1.024	Morning	11

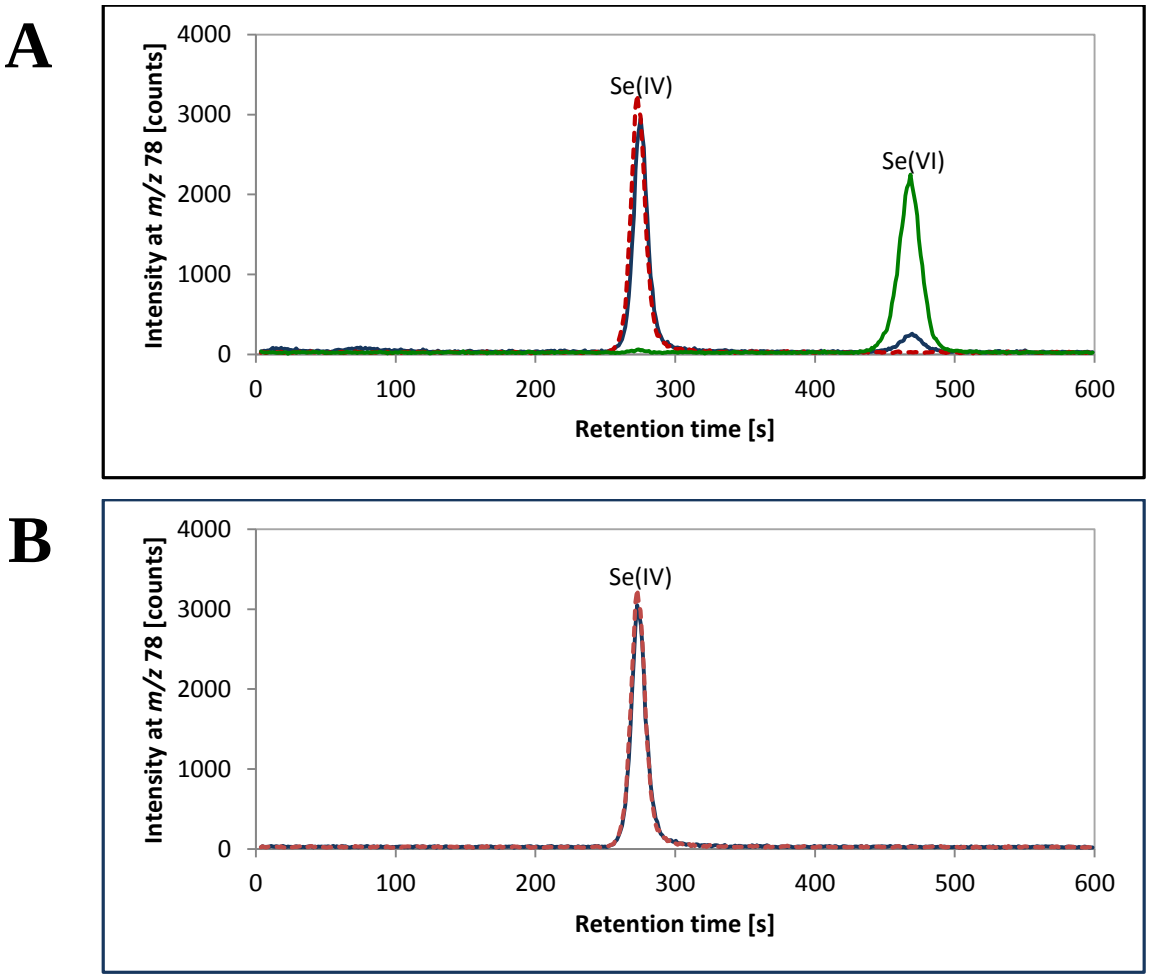

Figure 6: HPLC/ICPMS chromatograms of the commercially available selenium standards (column: PRP-X100 (5 µm, 4.6 x 150 mm); mobile phase: 20 mM malonate pH 9.5; flow rate: 1.0 mL/min; temperature: 30 °C; injection volume: 20 µL); (A) blue line: single-element-standard, (B) blue line: multi-element-standard; red line: selenite; green line: selenate

4.3 Optimization of FI/ICPMS

4.3.1 Carrier solution

The pH of human urine is usually between 4.5 and 8.0. [74] Hence, the use of a carrier solution in this range minimizes the risk of precipitation during measurements. Furthermore, the use of the same carrier solution for total measurements and speciation analysis would be advantageous. A mobile phase frequently used in our lab for the determination of selenium compounds in urine by reversed-phase HPLC/ICPMS is 20 mM ammonium formate 3 % MeOH pH 3.0. Hence, it was investigated first. 20 mM malonate was used to cover a wider range of pH values (pH 3.0, 6.0 and 9.5, with and without 3 % MeOH). Nitric acid (0.005 M in 10 % MeOH, pH 2.3) was also checked as a possible carrier solution because its use was reported in the literature (Table 1). Different selenium species, which might be contained in

body fluids (selenite, selenate, selenosugar **1**, TMSe, SeMet, MeSeCys) and the single-element-standard were investigated. Furthermore, three urine samples were measured.

The use of 0.005 M nitric acid in 10 % MeOH (pH 2.3) as carrier solution resulted in broad peaks and too high recoveries (> 120 %) for urine samples compared to the results of the conventional method. Furthermore, the low pH might be problematic for steel parts of the HPLC system. Therefore, nitric acid was not further tested.

Solutions of the different selenium species (20 µg Se/L each) and urine 3 were injected into 20 mM ammonium formate pH 3.0 or 20 mM malonate pH 3.0 with and without 3 % methanol to check the influence of methanol on the peak shape. To obtain comparable results, all measurements were carried out with Ar/CO_2 as optional gas. No significant differences in the peak shape could be observed for both carrier solutions, the duplicate injections matched each other well, but more carry-over was found in the presence of methanol (Figure 7).

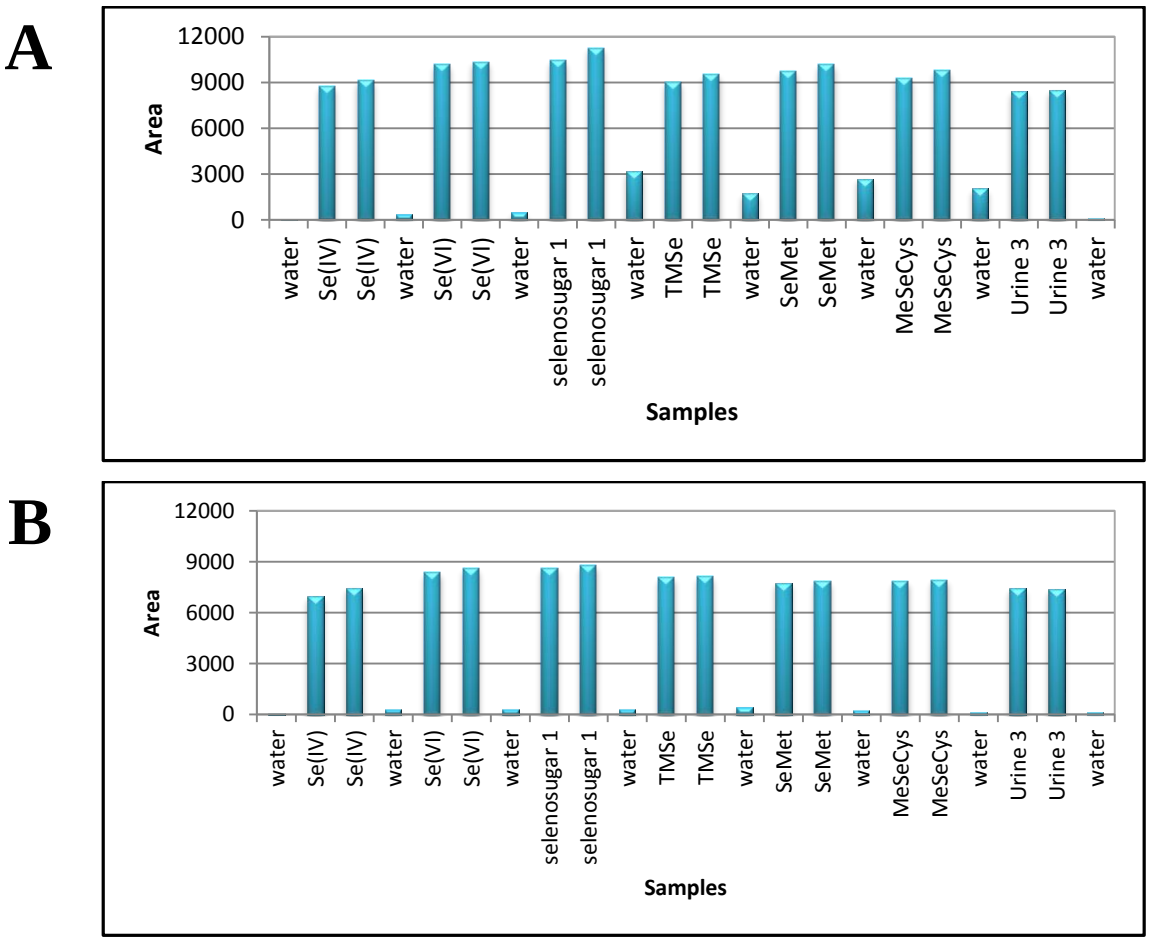

Figure 7: Influence of methanol in the carrier solution on carry-over (carrier solution: 20 mM ammonium formate pH 3.0; flow rate: 0.4 mL/min; integration time: 0.3 s/point; injection volume: 2 µL); (A) with methanol, (B) without methanol

However, the response of the selenium species did not match each other when carrier solutions at pH 3.0 were used (Figure 8). This could be explained by the broader peak shapes at pH 3.0, especially in the case of selenate (Figure 9). Further, the obtained signal at pH 3.0 is lower than at a higher pH. Therefore, carrier solutions (20 mM malonate) at pH 6.0 and 9.5 were investigated, which resulted in comparable peak areas. Due to the pH of human urine, 20 mM malonate pH 6.0 was chosen for further optimization.

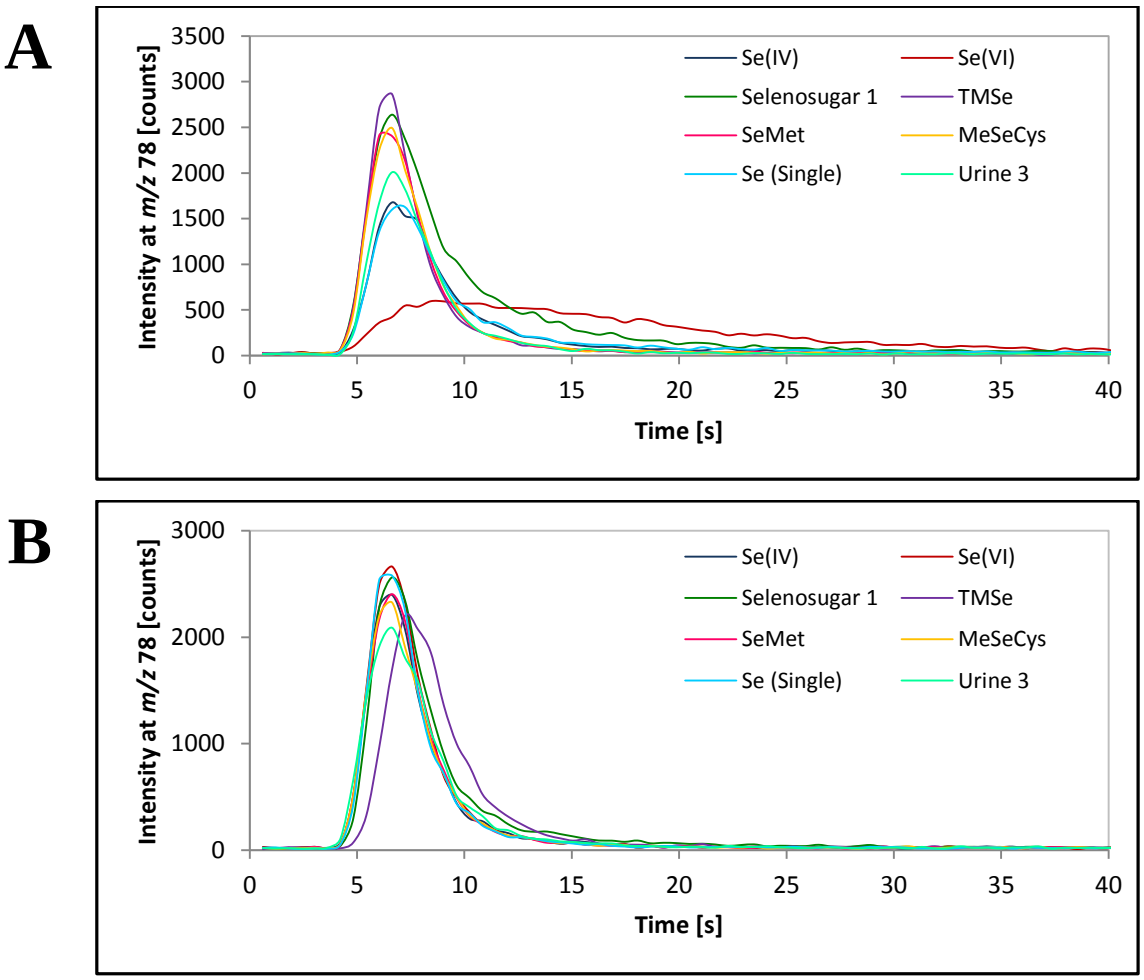

Figure 8: FI/ICPMS response of the different selenium species (flow rate: 0.4 mL/min; integration time: 0.3 s/point; injection volume: 2 µL); (A) with 20 mM malonate pH 3.0, (B) with 20 mM malonate pH 6.0

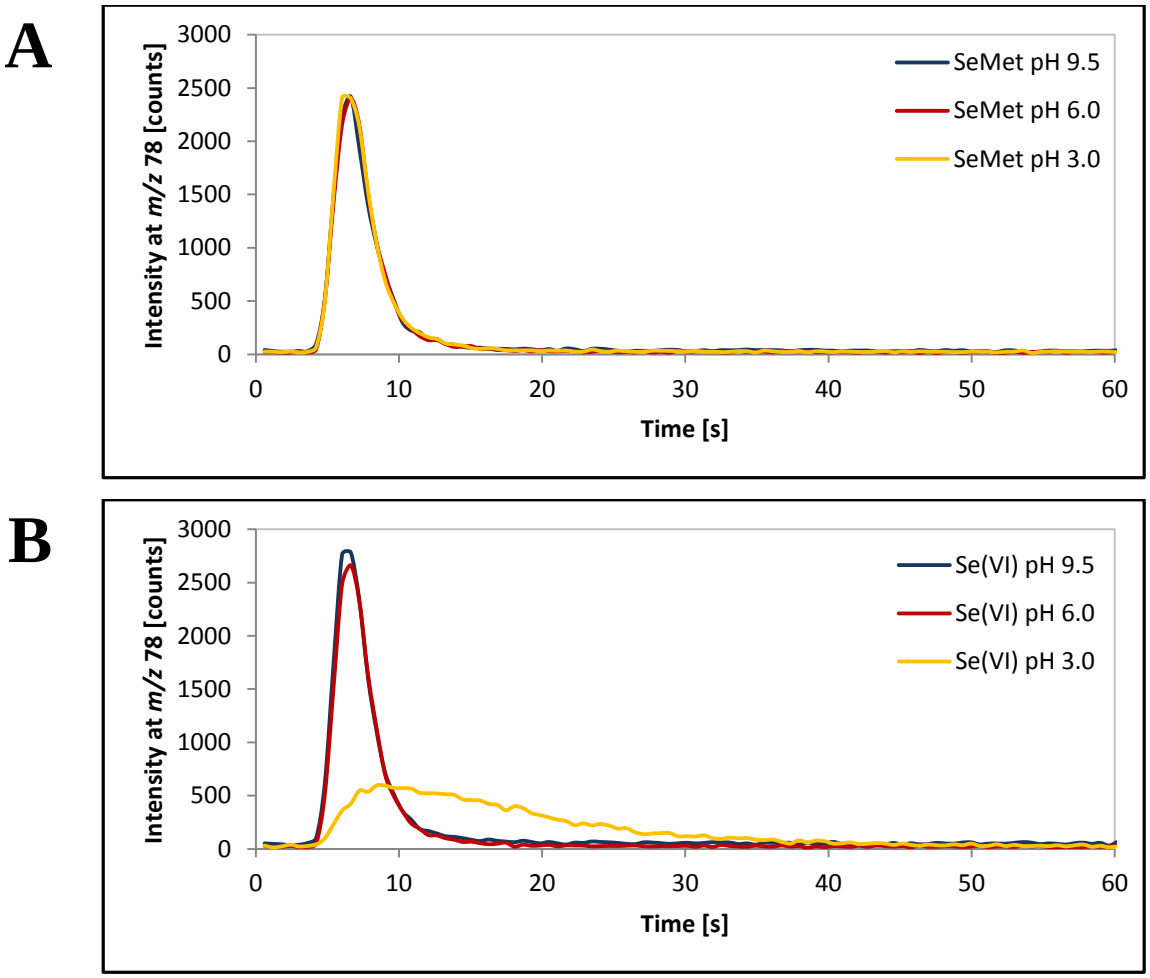

Figure 9: Influence of the pH of the carrier solution on the peak shape of the FI/ICPMS signal (flow rate: 0.4 mL/min; integration time: 0.3 s/point; injection volume: 2 µL); (A) SeMet (20 µg Se/L), (B) Se(VI) (20 µg Se/L); blue line: 20 mM malonate pH 9.5; red line: 20 mM malonate pH 6.0; yellow line: 20 mM malonate pH 3.0

4.3.2 Flow splitting

Signal suppression was observed for the determination of selenium in the urine matrix. Signal suppresion can be minimized, when only a part of the matrix reaches the ICPMS, for example by reducing the injection volume. However, the injection volume cannot be reduced arbitrarily because the uncertainty of the injection volume becomes larger at lower injection volumes. Furthermore, the obtained signal decreases and, thus, accurate evaluation would not be possible anymore (see *Chapter 4.3.5*). This would result in higher LODs. Another possibility to reduce the sample matrix is dilution of the urine samples, which is time-consuming. Flow splitting is a good alternative to minimize matrix effects at acceptable injection volumes without additional time-consuming sample preparation steps. Another advantage compared to measurements without flow splitting was, that no carry-over was detected when carrier solutions containing methanol were used with flow splitting.

First, flow splitting was performed by using the set-up which is also routinely applied for conventional total measurements in our lab to achieve faster transport of the sample from the autosampler to the ICPMS. In this set-up the carrier solution enters a T-piece, where one part of the solution is directed to the waste, whereas the other part is introduced into the ICPMS via the nebulizer pump. Due to the lower signal suppression, better agreement with total selenium concentrations determined with the conventional method was obtained (Table 7). Standard addition improved the agreement and yielded results close to the target values. With external calibration, many samples can be measured without additional expenditure of time, but matrix effects are not compensated. To eliminate matrix effects, standard addition can be used for quantification, but this calibration type is associated with higher expenditure of time as an own standard addition experiment must be performed for each sample. [75] Hence, the further use of standard addition was not considered.

Due to the large dead volume of the total-set-up, broad peaks were obtained. Therefore, a passive splitter adjusted to the same split ratio (35 : 65) was used for flow splitting instead of the total-set-up, which resulted in narrower peak shapes because of the lower dead volume (Figure 10). This results in lower LODs and shorter analysis times.

Table 7: Total selenium concentration in urine obtained by FI/ICPMS with or without flow splitting (n = 3 injections)

Sample	FI/ICPMS [µg Se/L]	Conventional method [µg Se/L]	Agreement with the conventional method [%]
without flow splitting			
Urine 3 (external calibration)	16.0 ± 0.1	21.0 ± 0.8	76
Urine 3 (standard addition)	19.9 ± 0.8	21.0 ± 0.8	95
with flow splitting (total-set-up)			
Urine 3 (external calibration)	18.0 ± 0.1	21.0 ± 0.8	86
Urine 3 (standard addition)	20.7 ± 0.4	21.0 ± 0.8	99

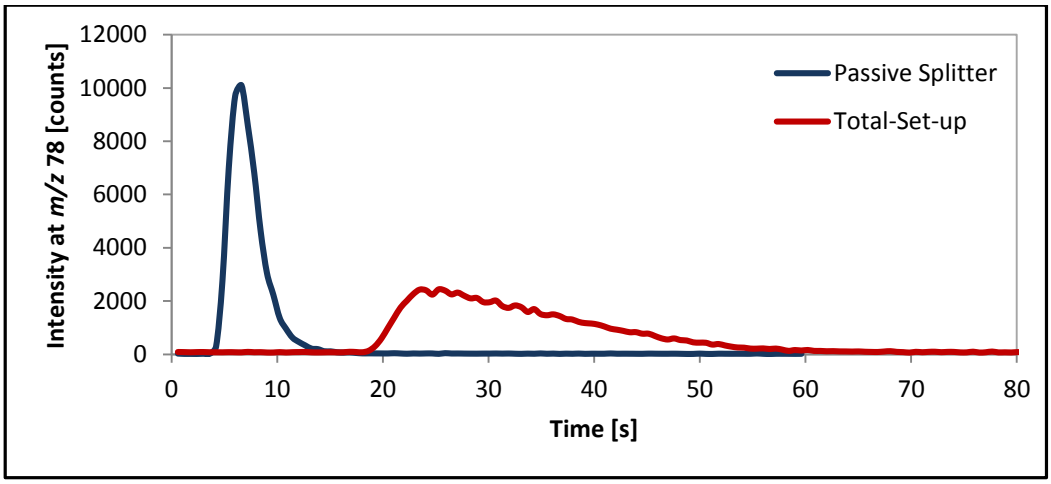

Figure 10: Comparison of the FI/ICPMS signals obtained for the single-element-standard (20 µg Se/L) with the passive splitter or with the total-set-up (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 35 : 65; flow rate: 1.0 mL/min; integration time: 0.3 s/point; injection volume: 20 µL); blue line: passive splitter; red line: total-set-up

Influence of the split ratio

Different split ratios were tested regarding their effect on the results for the urine samples (Figure 11). Better agreement with the results obtained by conventional measurement can be obtained by using lower split ratios, because less matrix reaches the detector, resulting in less signal suppression. However, there was hardly any difference in the quantitative results between a split ratio of 10 : 90 and 15 : 85 (Figure 11), but the signal was broader and smaller when a split ratio of 10 : 90 was used instead of 15 : 85 (Figure 12). Therefore, a split ratio of 15 : 85 was applied in further experiments.

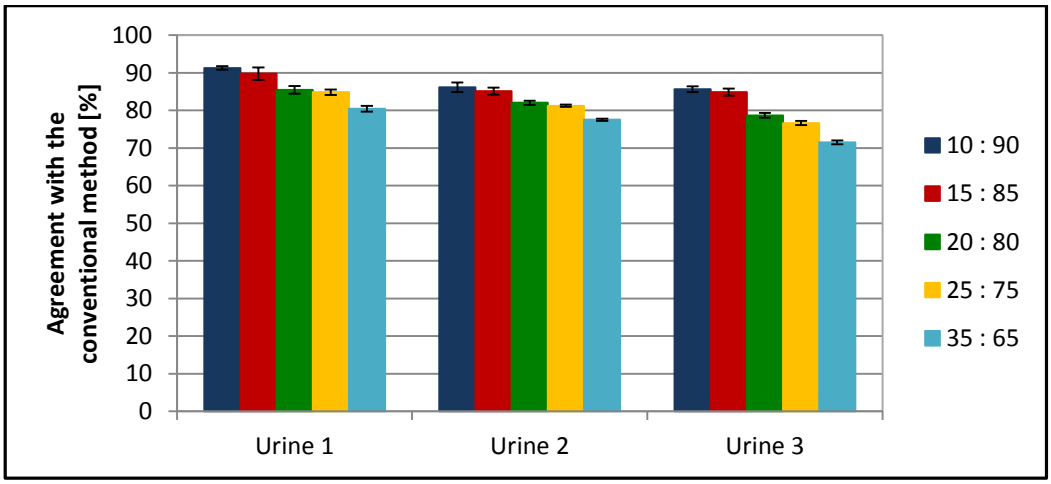

Figure 11: Influence of the split ratio on the agreement of the FI/ICPMS results with the results of the conventional method (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; flow rate: 1.0 mL/min; integration time: 0.3 s/point; injection volume: 20 µL; n = 3 injections)

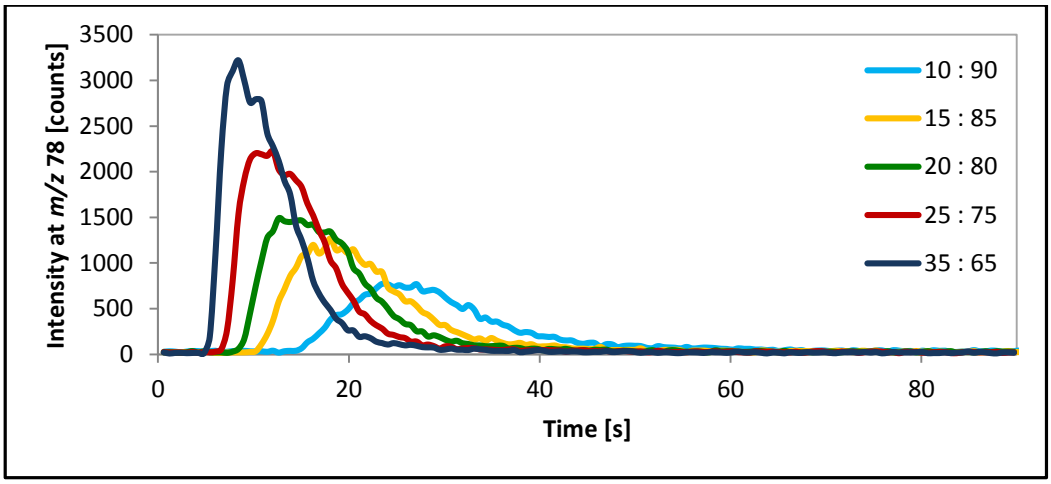

Figure 12: Comparison of the FI/ICPMS signal of urine 3 with different split ratios (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; flow rate: 1.0 mL/min; integration time: 0.3 s/point; injection volume: 20 µL)

4.3.3 Flow rate

The influence of the flow rate was tested using flow splitting (15 : 85) of the carrier solution. *Figure 13* shows the signals for three different flow rates. Peaks were broader at a flow rate of 1.0 mL/min than of 1.5 mL/min because the sample reaches the detector more quickly at higher flow rates, which minimizes diffusion of the analyte. However, there was no difference between the peak shapes obtained at a carrier solution flow rates of 1.5 mL/min and 2.0 mL/min. Since an increase in the flow rate generally resulted in a decrease of the peak area for the analyte (Figure 13) a flow rate of 1.5 mL/min was chosen for further optimization.

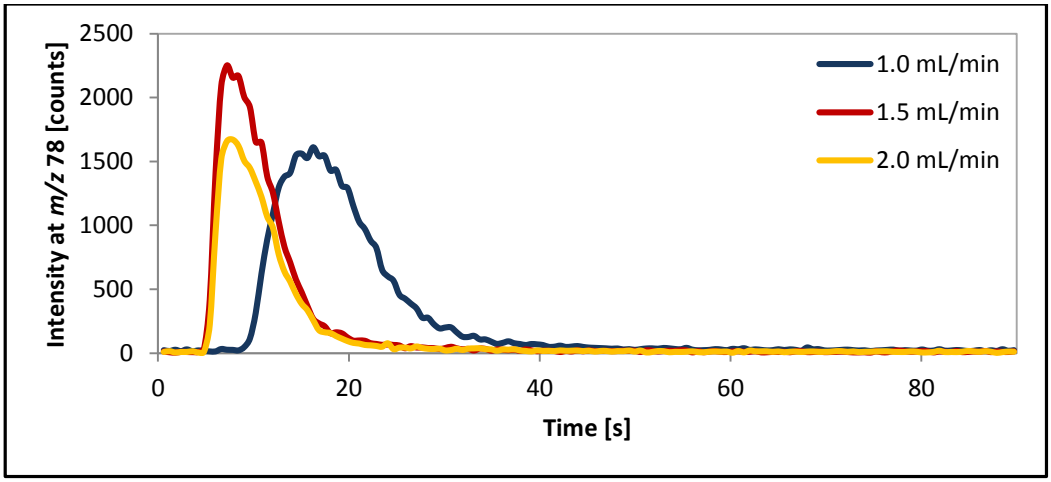

Figure 13: Comparison of the FI/ICPMS signal of urine 3 for different flow rates of the carrier solution (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 15 : 85; integration time: 0.3 s/point; injection volume: 20 µL); blue line: 1.0 mL/min; red line: 1.5 mL/min; yellow line: 2.0 mL/min

4.3.4 Integration time

To optimize the peak shape, different integration times were tested. Therefore, a single-element-standard (1 µg Se/L) was measured at different integration times (0.1 s/point, 0.2 s/point and 0.3 s/point). There are more points over the FIA curve when a lower integration time is used, which results in better peak shapes. Disadvantageous, however, is that a lower integration time resulted in a lower signal, but a concentration of 1 µg Se/L could still be evaluated (Figure 14) using an integration time of 0.1 s/point.

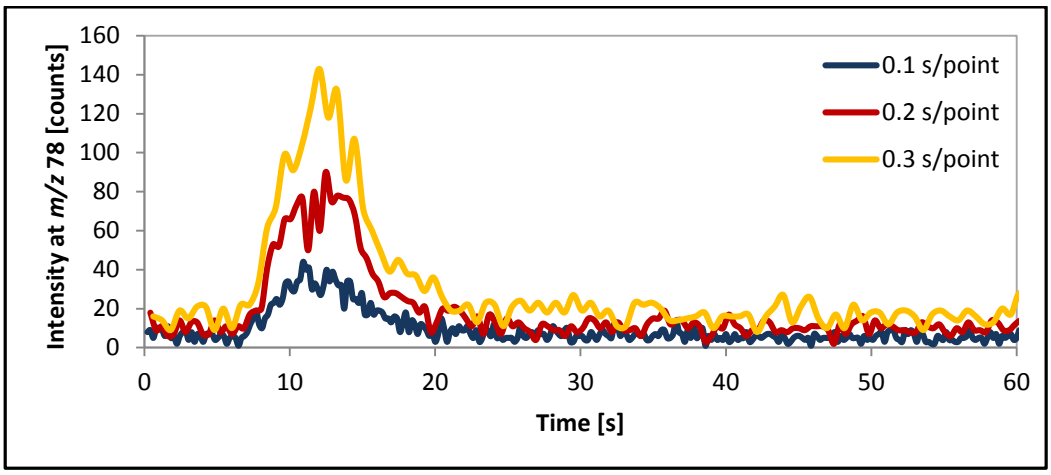

Figure 14: Influence of the integration time on the peak shape of the FI/ICPMS signal (1 µg Se/L single-element-standard) (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 15 : 85; flow rate: 1.5 mL/min; injection volume: 20 µL); blue line: 0.1 s/point; red line: 0.2 s/point; yellow line: 0.3 s/point

4.3.5 Injection volume

Injection volumes of 1 µL, 2 µL, 5 µL, 10 µL, 15 µL and 20 µL were compared to investigate the influence of this parameter on signal suppression. The signal of the lowest calibration standard (1 µg Se/L) could not be evaluated any more at injection volumes of 1 or 2 µL (Figure 15). Furthermore, the reproducibility of the injection volume is slightly worse at < 5 µL (< 1 % RSD of peak area) than of injection volumes between 5 and 100 µL (< 0.5 % RSD of peak area). Therefore, injection volumes of at least 5 µL are preferable. [76] In contrast, the matrix suppresion for urine was strong, when injection volumes of 20 µl were used (Figure 11). Therefore, 1 µL and 20 µL were not further tested. To check the influence of the urinary matrix, different urine samples were measured. Deviations from results obtained with the conventional method are reduced at lower injection volumes because of lower signal suppression by the matrix (Figure 16). Best recoveries were obtained by using an injection volume of 2 µL and 5 µL, but due to the slightly better precision in most cases and the low signal of the standard containing 1 µg Se/L at an injection volume of 2 µL, 5 µL were considered as optimum. This injection volume is considerably lower than injection volumes of FI/ICPMS reported in the

literature (Table 1). The small injection volume together with the considerable matrix reduction by the application of flow splitting explains the satisfactory agreement of the FI/ICPMS results with the target values of the conventional method even without the use of standard addition calibration or an internal standard.

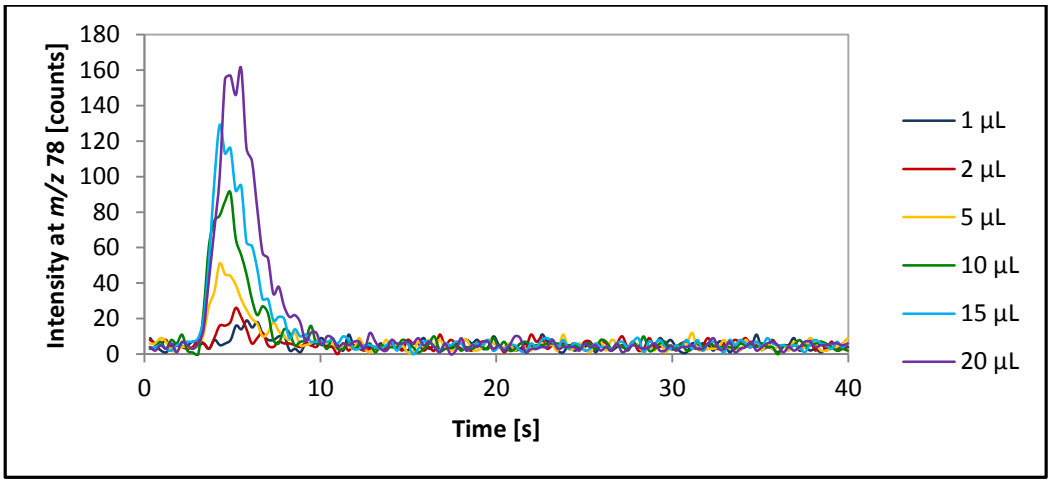

Figure 15: Comparison of the FI/ICPMS signal (1 µg Se/L single-element-standard) at different injection volumes (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 15 : 85; flow rate: 1.5 mL/min; integration time: 0.1 s/point)

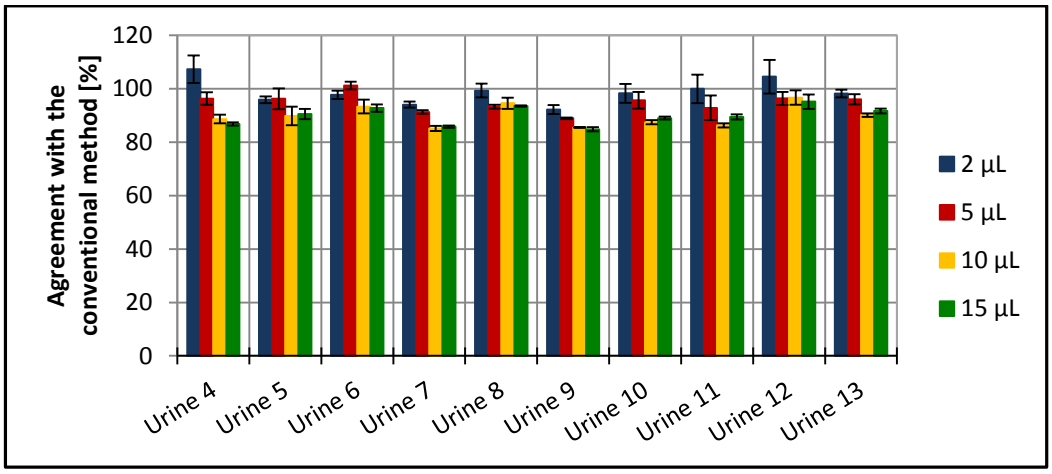

Figure 16: Influence of the injection volume on the agreement of the FI/ICPMS results with the results of the conventional method (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 15 : 85; flow rate: 1.5 mL/min; integration time: 0.1 s/point; n = 3 injections)

4.3.6 Internal standard

To correct for signal fluctuation through instrument instability and signal suppression caused by the matrix, ^{72}Ge was tested as internal standard. Germanium was selected because it was not expected to be present in urine at higher concentrations and has been successfully applied also with our conventional method. Furthermore, germanium has already been reported in the

literature as internal standard for the determination of total selenium in urine by FI/ICPMS (Table 1). [68] Compared to the other internal standard reported in the literature, rhodium, [33, 69, 71] germanium shows a mass-to-charge ratio more similar to that of the target analyte, selenium. Two different ways of germanium addition were investigated (Figure 17):

- the internal standard was added to the carrier solution; the signal of the internal standard was constantly high and decreased, when a sample with high matrix reached the detector
- the internal standard was added to the samples by an injector program performed by the autosampler thus resulting in a germanium peak; the area of the germanium peak decreased, when a sample with a high matrix reached the detector

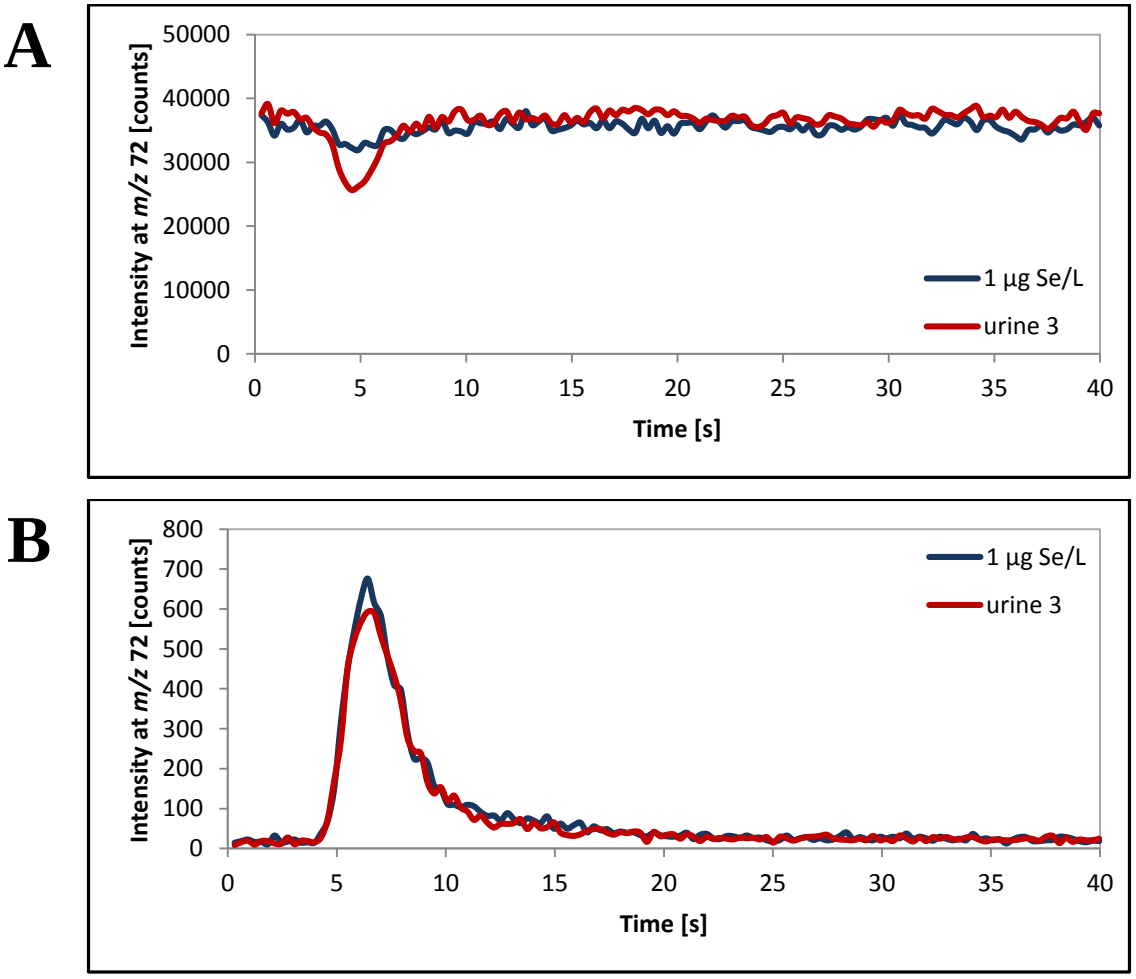

Figure 17: FI/ICPMS signal of the internal standard germanium with different methods of internal standard addition (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 15 : 85; flow rate: 1.5 mL/min; integration time: 0.1 s/point; injection volume: 5 µL); (A) internal standard (50 µg Ge/L) added to the carrier solution, (B) injection program (1 µL of 50 µg Ge/L added to 5 µl of sample); blue line: 1 µg Se/L (single-element-standard); red line: urine 3

The results obtained by internal standard correction were compared with results obtained without internal standard correction. For both methods internal standard correction resulted in

concentrations closer to the target values (Figure 18). Furthermore, standard deviations were higher, when the injection program was used than when the internal standard was in the carrier solution, because in the latter case each point of the FIA curve was individually corrected. Another reason for the higher standard deviation obtained with the injection program is that a very low volume of the internal standard (1 µL) was added to the sample to avoid dilution of the urine and, hence, lower signal (see also *Chapter 4.3.5*). A further advantage is the shorter measurement time when using the internal standard in the carrier solution – more time is required for injection when the injection program is used.

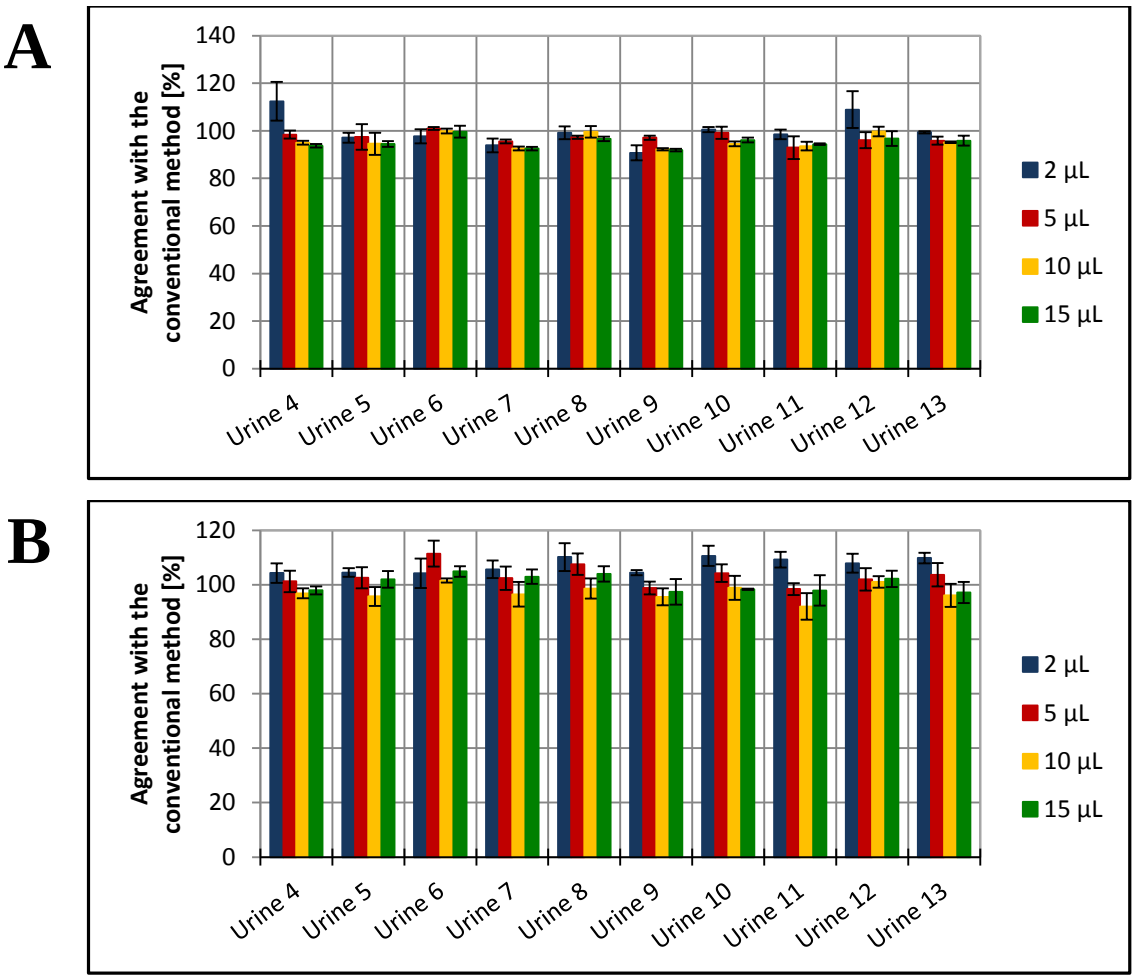

Figure 18: Influence of internal standard correction at different sample injection volumes (carrier solution: 20 mM malonate 3 % MeOH pH 6.0; split ratio: 15 : 85; flow rate: 1.5 mL/min; integration time: 0.1 s/point; n = 3 injections); (A) internal standard in the carrier solution (50 µg Ge/L), (B) injection program (addition of 1 µL of 50 µg Ge/L with the HPLC autosampler)

Good results (less than 10 % deviation from the target concentration) were obtained for all injection volumes when the internal standard was used. Based on the results obtained without internal standard correction (see *Chapter 4.3.5*), an injection volume of 5 µL was preferred. Therefore, the measurement parameters for the optimized FI/ICPMS method for the

determination of total selenium in human urine were as follows: carrier solution: 20 mM malonate 3 % MeOH pH 6.0 containing 50 µg Ge/L for internal standard correction; split ratio: 15 : 85; flow rate: 1.5 mL/min; integration time: 0.1 s/point; injection volume: 5 µL.

4.4 Analytical Performance

The method performance characteristics of the optimized method were determined using the optimized conditions.

Germanium-corrected calibration curves showed linearity in the investigated concentration range of 1 to 100 µg Se/L with a correlation coefficient (r^2) better than 0.999.

Accuracy of the developed FI/ICPMS method was checked in three different ways:

(i) the certified reference material NIES No. 18 Human Urine was measured

(ii) the selenium concentrations in different urine samples were compared to results obtained with the conventional method

(iii) urine samples were spiked with selenium standard solutions and recoveries were determined

The total selenium concentration determined in the certified reference material NIES No. 18 Human urine was 61.4 ± 0.4 µg Se/L (n = 3), which matched the certified range of 59 ± 5 µg Se/L.

For different urine samples (total selenium concentrations determined with the conventional method between 1.2 µg Se/L and 20.0 µg Se/L) the agreement with the target concentrations determined by the conventional method was – with one exception – between 93 % and 101 % (mean: 95 %). (Table 8) There was no obvious correlation between specific gravity as measure for total dissolved solids and the deviation from the target value. Surprisingly, the only sample showing a rather large deviation (urine 15) had a low specific gravity indicating less matrix than in most of the other urine samples. The reasons for this result remain unknown. However, it has to be noted that the total selenium concentration in this sample was at the lower limit of typical selenium concentrations in human background urine.

Table 8: Comparison of results obtained by the developed FI/ICPMS method with results from the conventional method (n = 3 injections or digests, respectively)

	FI/ICPMS [µg Se/L]	Conventional method [µg Se/L]	Agreement with the conventional method [%}	Specific gravity
Urine 4	7.4 ± 0.2	7.6 ± 0.1	98	1.011
Urine 5	10.4 ± 0.6	10.7 ± 0.1	97	1.012
Urine 6	6.5 ± 0.1	6.5 ± 0.1	101	1.011
Urine 7	19.1 ± 0.2	20.0 ± 0.1	96	1.020
Urine 8	12.6 ± 0.1	12.9 ± 0.1	97	1.012
Urine 9	18.1 ± 0.2	18.6 ± 0.2	97	1.015
Urine 10	9.8 ± 0.3	9.9 ± 0.3	99	1.013
Urine 11	7.4 ± 0.4	7.9 ± 0.1	93	1.011
Urine 12	7.5 ± 0.2	7.8 ± 0.2	96	1.007
Urine 13	9.9 ± 0.2	10.3 ± 0.2	96	1.010
Urine 14	1.1 ± 0.1	1.2 ± 0.1	94	1.002
Urine 15	1.8 ± 0.1	2.5 ± 0.1	71	1.003
Urine 16	1.8 ± 0.1	1.9 ± 0.2	94	1.002

Spiking recoveries were determined by analyzing two different urine samples in triplicate before and after spiking with about the originally contained amount of selenium in the form of the single-element standard solution. Recoveries were calculated in % by comparing the original sample with the spiked sample. Spiking recoveries were 103 % and 88 % for urine 6 and 7, respectively. The reason for the difference in the recoveries of both urine samples might be that urine 7 was very rich in matrix (specific gravity: 1.020), whereas the specific gravity of urine 6 was lower (1.011).

Intra-day and inter-day precision were determined with 13 and 10 different urine samples, respectively (Table 9). Intra-day precision of the developed method for the determination of selenium in urine by FI/ICPMS varied, depending on the urine sample, from 1 to 7 % RSD with better precision at total selenium concentrations above 5 µg Se/L. Inter-day precision was slightly higher (3 to 7 % RSD) compared to intra-day precision for the same samples (1 to 4 % RSD). In general, the precision of the optimized method was comparable to FI/ICPMS methods published in the literature. [33, 68, 71]

Table 9: Intra-day and inter-day precision of the optimized FI/ICPMS method (measured on three or four different days, n = 3 injections on each day)

Sample	Concentration [µg Se/L]	Intra-Day RSD [%]	Inter-Day RSD [%]
Urine 14*	1.2 ± 0.1	6.9	-
Urine 16*	1.9 ± 0.2	5.3	-
Urine 15*	2.5 ± 0.1	4.7	-
Urine 6	6.5 ± 0.1	1.8	5.9
Urine 4	7.6 ± 0.1	3.3	5.3
Urine 12	7.8 ± 0.2	2.7	3.4
Urine 11	7.9 ± 0.1	4.3	4.7
Urine 10	9.9 ± 0.3	2.4	5.2
Urine 13	10.3 ± 0.2	2.7	6.1
Urine 5	10.7 ± 0.1	3.3	6.7
Urine 8	12.9 ± 0.1	2.3	2.5
Urine 9	18.6 ± 0.2	0.9	5.5
Urine 7	20.0 ± 0.1	1.2	3.0

**10 injections on one day*

The method’s limit of detection (LOD) and limit of quantification (LOQ) were determined in the urinary matrix by using urine samples with low total selenium concentrations (1.2 – 2.5 µg Se/L). The LOD in urine was calculated by the 3*σ method, whereas the LOQ was determined by the 10*σ method to be 0.2 µg Se/L and 0.8 µg Se/L, respectively, which is in the range of other published methods (Table 1). Both, the precision and LOQ were considered as sufficient for the determination of total selenium in urine samples.

4.5 Stability of the urine samples

It is known that selenosugar **1**, the major selenium metabolite in human urine, is not stable when urine is stored at 4 °C. [43] With time, it is transformed to volatile compounds. Because these volatile compounds are transported more efficiently to the plasma, they give a higher response and overestimated results. To explore how long urine can be stored and still gives accurate results, the stability of different urine samples stored in the refrigerator was investigated. During storage, the concentration of selenosugar **1** in the tested urine samples decreased slightly (Table 10). A more pronounced decrease was found in the urine sample spiked with selenosugar **1**. Before storage, no volatile compounds were detected, whereas after 4 days storage volatile compounds were found (Figure 19), which resulted in higher response and

hence slightly higher selenium concentrations in some of the samples measured by FI/ICPMS (Table 11).

Table 10: Concentration of selenosugar **1** in urine samples before and after storage of 4 and 14 days

Sample	Concentration of selenosugar 1 [µg Se/L]		
	day 0	day 4	day 14
Urine 4	0.3	0.2	0.2
Urine 6	0.3	0.2	0.2
Urine 6 spiked	-	28.0	19.5
Urine 7	1.5	1.1	1.0
Urine 9	1.1	0.9	0.9
Urine 17	4.1	3.6	3.1
Urine 18	1.6	1.0	1.0

After 14 days storage no volatile selenium compounds could be detected by HPLC/ICPMS (with exception of spiked urine 6), which explains the slight decrease of total selenium concentrations determined by FI/ICPMS towards day 14. Obviously, the volatile selenium compounds detected on day 4 had escaped from the solution until day 14 probably due to shaking of the tubes before analysis. Although, corresponding to previous results, [43] most of the urine samples tested yielded the same results in FI/ICPMS over 2 weeks, storage for longer than 4 days at 4 °C is not recommended, especially, if urine contains high concentrations of selenosugar **1**. Calibration standards in the concentration range from 2 – 50 µg Se/L were stable for 14 days at 4 °C in 15 mL tubes.

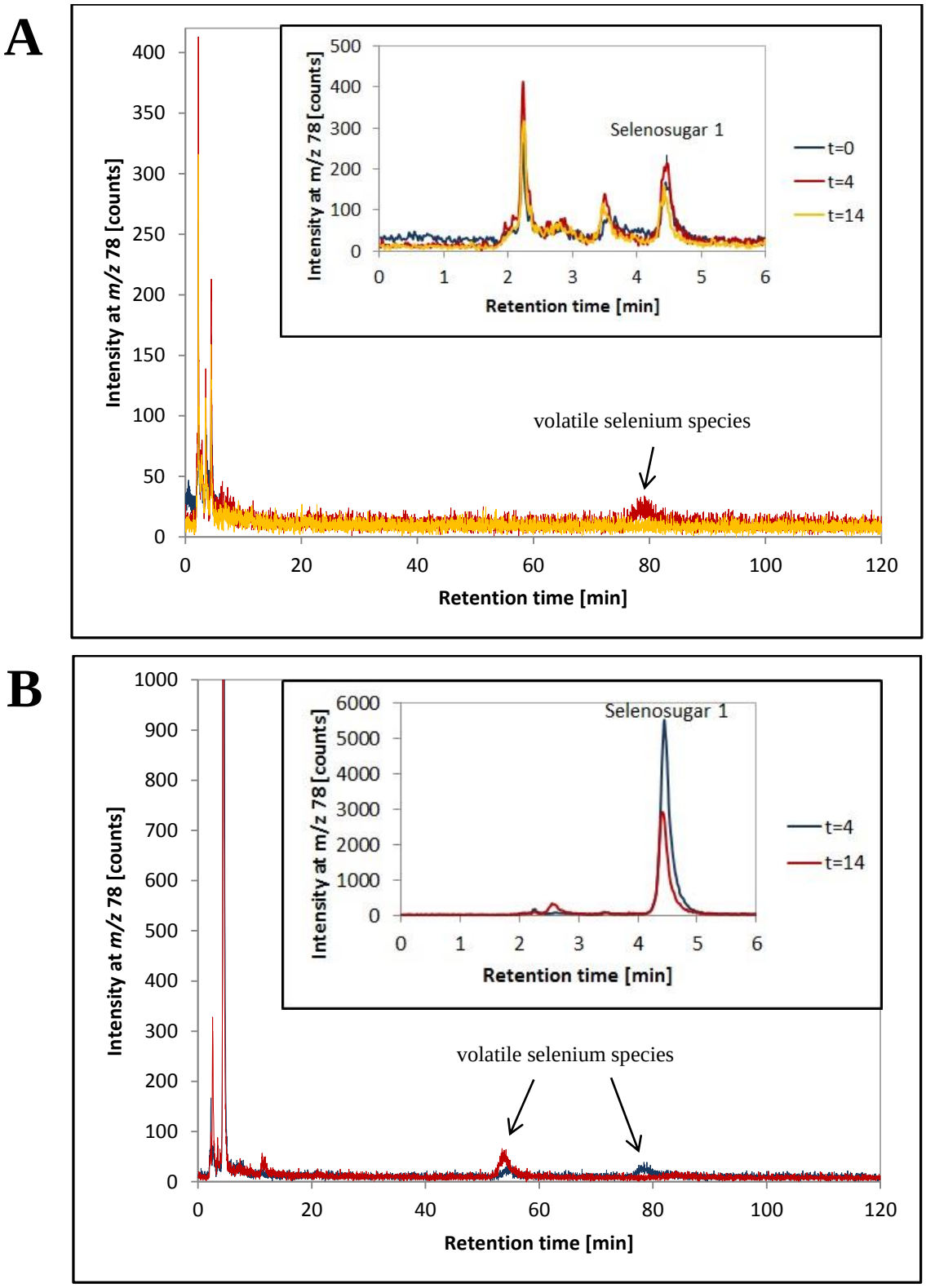

Figure 19: HPLC/ICPMS chromatograms of urinary selenium species before storage (t=0) and after 4 days (t=4) and 14 days (t=14) (column: Atlantis® dC18 (5 µm, 4.6 x 150 mm); mobile phase: 20 mM ammonium formate 3 % MeOH pH 3.0; flow rate: 1.0 mL/min; column temperature: 30 °C; injection volume: 20 µL); (A) urine 17, (B) urine 6 spiked with 30 µg Se/L as selenosugar **1** before storage; blue line: t=0 days; red line: t=4 days; yellow line: t=14 days

Table 11: Stability of urine samples after storage at 4 °C for up to 14 days

Sample	Total selenium concentration [µg/L]								
	Target*	day 0**	day 1**	day 2**	day 3**	day 4**	day 7**	day 10**	day 14**
Urine 4	7.6 ± 0.1	7.3 ± 0.2	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.3 ± 0.1	7.2 ± 0.2	7.4 ± 0.2	7.0 ± 0.1
Urine 6	6.5 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.3 ± 0.2	6.6 ± 0.2	6.8 ± 0.3	7.0 ± 0.7	6.7 ± 0.1
Urine 6 spiked	36.9 ± 0.2	36.0 ± 0.4	35.6 ± 0.5	36.0 ± 0.1	36.7 ± 0.4	36.7 ± 0.2	41.0 ± 0.6	43.4 ± 0.8	39.2 ± 0.2
Urine 7	20.0 ± 0.1	19.2 ± 0.1	19.0 ± 0.5	18.9 ± 0.2	18.4 ± 0.2	19.2 ± 0.2	18.5 ± 0.3	18.9 ± 0.5	18.9 ± 0.2
Urine 9	18.6 ± 0.2	17.4 ± 0.2	17.2 ± 0.4	17.7 ± 0.4	17.5 ± 0.3	17.9 ± 0.4	17.0 ± 0.3	17.0 ± 0.3	16.4 ± 0.3
Urine 17	10.6 ± 0.3	10.5 ± 0.1	10.3 ± 0.2	10.3 ± 0.4	10.4 ± 0.4	10.8 ± 0.2	11.1 ± 0.3	10.9 ± 0.1	10.5 ± 0.3
Urine 18	29.8 ± 0.1	28.5 ± 0.3	28.1 ± 0.3	27.7 ± 0.1	28.2 ± 0.4	28.9 ± 0.2	28.9 ± 0.7	28.6 ± 1.0	28.1 ± 0.1

** determined with the conventional method*

*** determined with the optimized FI/ICPMS method*

5 Concluding Comments

The developed FI/ICPMS method is suitable for the determination of total selenium in urine samples. LOD, LOQ, accuracy, and precision meet the requirements for urine even at background concentrations in the low µg Se/L range. Time-consuming sample preparation can be avoided, because matrix effects are minimized by low injection volumes, flow splitting, and internal standard correction. Hence, urine can be analyzed directly only after filtration. Urine can be stored at least for 4 days at 4 °C before analysis. Simple sample preparation together with an analysis time of about 2 minutes/sample, as well as the low requirement for sample volume makes the method very useful particularly for large numbers of samples and samples, for which only limited volumes are available. In the future, the applicability to other biological fluids, such as blood, serum, and plasma should be investigated.

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7 Appendix

Typical ICPMS settings

Plasma Parameters			
RF Power	1550	[1550]	W
RF Matching	1.73	[1.73]	V
Smpl Depth	8.0	[8.0]	mm
Torch-H	0.1	[0.1]	mm
Torch-V	0.7	[0.7]	mm
Carrier Gas	0.75	[0.75]	L/min
Makeup Gas	0.00	[0.00]	L/min
Optional Gas	12.0	[12.0]	%
Nebulizer Pump	0.50	[0.50]	rps
Sample Pump	0.00	[0.00]	rps
S/C Temp	2	[2]	degC
Ion Lenses			
Extract 1	2.5	[2.5]	V
Extract 2	-115.0	[-115.0]	V
Omega Bias-ce	-18	[-18]	V
Omega Lens-ce	-1.6	[-1.6]	V
Cell Entrance	-20	[-20]	V
QP Focus	-9	[-9]	V
Cell Exit	-40	[-40]	V
Q-Pole Parameters			
AMU Gain	129	[129]	
AMU Offset	126	[126]	
Axis Gain	0.9997	[0.9997]	
Axis Offset	-0.02	[-0.02]	
QP Bias	-16.0	[-16.0]	V
Octpole Parameters			
OctP RF	200	[200]	V
OctP Bias	-18.0	[-18.0]	V
Reaction Cell			
Reaction Mode	ON	[ON]	
H2 Gas	3.5	[3.5]	mL/min
He Gas	0.0	[0.0]	mL/min
Optional Gas	0	[0]	%
Detector Parameters			
Discriminator	6.1	[6.1]	mV
Analog HV	2100	[2100]	V
Pulse HV	1690	[1690]	V

Figure A. 1: typical ICPMS settings in the H_2 mode

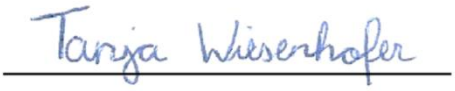
The inner diameter of the torch was 1 mm.

For measurements using the conventional method and FI/ICPMS using the total-set-up the nebulizer pump was set to 0.11 rps instead of 0.50 rps.

8 Affidavit

I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly indicated all material which has been quoted either literally or by content from the sources used. The text document uploaded to TUGRAZonline is identical to the present master‘s thesis dissertation.

Graz, the 25.03.2015



Tanja Wiesenhofer