SELENIUM DETERMINATION IN WHOLE BLOOD, PLASMA AND SELENOPROTEIN P BY HYDRIDE GENERATION ATOMIC FLUORESCENCE SPECTROMETRY†

Darja Mazej, Ingrid Falnoga, Vekoslava Stibilj

Jožef Stefan Institute, Ljubljana, Slovenia

†This paper is dedicated to Dr. Marjan Dermelj, on the occasion of his 70th birthday.

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Abstract

Selenium was determined in whole blood, plasma and plasma protein fractions SeP and (GSH-Px+Alb) obtained by affinity chromatography. For the digestion of whole blood and plasma samples a simple digestion procedure using H_2SO_4, H_2O_2 and V_2O_5 in H_2SO_4 solution was developed, while for the decomposition of plasma protein fractions HNO_3 and H_2O_2 were sufficient. After both procedures detection of Se was made by HG-AFS and this combination gave reliable results. Dilution of the GSH-Px+Alb fraction was reduced by lyophilisation of pooled subfractions. The main advantages of the described procedures are low detection limit (0.2 ng Se/g of solution) and the large number of samples analysed in a short time as only two analyses are needed for determination of the percentage of Se in SeP in plasma sample.

The procedures developed were used to determine selenium in real samples from which an average mass fraction of selenium in whole blood of Slovenia residents of 87 ± 13 ng/g (n=43) and Se in SeP of 70 ± 9% (n=5) were found. Both values are comparable with literature data for the general population.

Introduction

The essential trace element selenium is of fundamental importance to human health. As a constituent of selenoproteins, selenium has an enzymatic role, best-known as an antioxidant scavenging free radicals, and as a catalyst for the production of active thyroid hormone.[1] About 35 selenoproteins have been identified, though for many of them their role has not been fully explained.[2] In mammalian plasma, selenium is mainly incorporated in three proteins, selenoprotein P (SeP), extracellular glutathione peroxidase (GSH-Px) and albumin (Alb). SeP is the major selenoprotein in plasma. All selenoproteins so far reported contain one atom of Se as selenocysteine per molecule except SeP, which contains ten atoms of Se as selenocysteine per molecule. The function of SeP is currently unknown, although several indirect lines of evidence suggest that SeP is a free radical scavenger or Se transporter.[3]

Estimation of human selenium status has become more and more important. Most frequently the measurement of selenium in whole blood and its fractions and GSH-Px activity have been used as a measure of Se status. In recent times the distribution between plasma proteins has become an important indicator and SeP as the major plasma protein has become an interesting marker of Se status. In this regard several separation procedures based on chromatography have been developed to separate SeP from GSH-Px and albumin. Affinity chromatography based on specific binding of SeP on sepharose–bound heparin has been most often used.[4,5] Deagen et al first described a method for the separation of three major selenium containing proteins in plasma using two small columns of heparin-Sepharose and reactive blue 2-Sepharose linked together in tandem. The first column was used to remove SeP, while albumin was retained on blue-Sepharose. GSH-Px passed through both columns. Selenium was determined spectrofluorimetrically.[4] Harrison et al made some changes to this procedure. The same columns were used independently of each other and smaller subfractions were collected to decrease the dilution effect of serial columns and joint fractions. Because a different detection system was used, electrothermal atomic absorption (ET-AAS), the type of buffer was changed from phosphate to acetate. Se determinations by ET-AAS were faster but less sensitive.[5]

Several detection techniques have been used for the determination of the total concentration of selenium in human plasma and whole blood, including neutron activation analysis (NAA), molecular fluorescence spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution mass spectrometry, ET-AAS, hydride generation atomic absorption spectrometry (HG-AAS) and hydride generation atomic fluorescence spectrometry (HG-AFS).[6] Various mixtures of nitric, perchloric and sulphuric acids with hydrogen peroxide in open or closed systems are often used for digestion of blood samples.[7,8] For determination of selenium in protein fractions after affinity chromatography where the concentrations of selenium are very low, the amount of fraction available is limited and the number of fractions is numerous, the choice of detector is narrower, mostly limited to ET-AAS and HG-AFS. For HG-AFS detection systems the digestion of samples is necessary.

The aim of this work was to develop and optimize a very simple, reliable and sensitive method for the determination of selenium in whole blood, plasma and plasma

protein fractions obtained after chromatographic separation. For the isolation of SeP from plasma, a published[5] separation procedure was used and partly modified regarding collection and preparation of the chromatographic fractions. Harrison et al determined Se in fractions directly by ET-AAS,[5] but we used digestion of fractions with nitric acid and hydrogen peroxide. The digestion procedure using sulfuric acid, hydrogen peroxide and V_2O_5 in H_2SO_4 for whole blood and plasma samples, which has already been published,[9] was also modified. Both digestion procedures were followed by HG-AFS detection.

Experimental

Blood and plasma samples

43 blood samples (2 x 7 mL) from residents of Slovenia were collected at the Clinical Centre, Ljubljana in Vacutainer tubes (Becton Dickinson) containing either no anticoagulant or Na- or Li- heparin. After cooling at room temperature (20 min), the sample aliquots were centrifuged (10 minutes, 2500 rpm). The resulting plasma samples were transferred to glass tubes with covers (Labco). One part of the plasma was stored at 4 ºC for not more than two days before separation. Blood and the other part of the plasma samples were stored at –20 ºC prior to Se determination.

Isolation of SeP from plasma by affinity chromatography

a) Reagents: In chromatographic separation ultra-pure water (Milli Q, Millipore, USA) and the following chemicals were used: NaCl (Merck, suprapur), CH_3COONH_4 (Merck, practopur), 90% CH_3COOH (Merck, suprapur), heparin (Krka, 5000 U/mL), 65% HNO_3 (Merck, suprapur).

Equilibrium buffer (0.02 mol CH_3COONH_4/L, pH 6.8): 1.54 g CH_3COONH_4 was dissolved in 1 L of Milli-Q water.

Elution buffer (0.02 mol CH_3COONH_4/L with 500 U/L heparin, pH 6.8): 5 mL heparin (5000 U/L) was diluted with 50 mL of equilibrium buffer.

Regeneration buffers (0.02 mol CH_3COONH_4/L with 0.5 mol/L NaCl, pH 5 and 10): 2.92 g NaCl was dissolved in 100 mL of equilibrium buffer, and the pH was adjusted with 5% acetic acid or 5% ammonium hydroxide.

b) Procedure: Proteins were separated in a glass column (30 cm x 1 cm, Econo, Bio-Rad) filled with 2 g of heparin-Sepharose CL-6B (Pharmacia). The flow rate through the column was 2.5 mL/min and was adjusted at the peristaltic pump (Ismatec, MCP 380) by

the choice of tubing size (Tygon, LFL). The column was equilibrated with 50 mL of equilibrium buffer. 2 g of plasma were diluted with equilibrium buffer (ratio 1:1) and applied to the column. GSH-Px + Alb (fraction A) was eluted from the column with 40 mL of equilibrium buffer. SeP (fraction B) bound to heparin-sepharose was then eluted with 50 mL of elution buffer and collected in 20 subfractions, 2.5 mL each. The column was regenerated with regeneration buffers, 16 mL of each and washed with equilibrium buffer (3 x 8 mL) before re-equilibration. Fraction A was lyophilised to 1/5 of its volume (24 hours, –52 °C, 0.030 mbar, freeze drier LOC-1, CHRIST) and then diluted to 10 g with Milli-Q water. 200 μL of 65% HNO_3 was added to subfractions of fraction B and fraction A. Fraction A and B were stored at –20 °C before selenium determination.

Selenium determination

a) Reagents: For preparation of solutions and sample treatment ultra-pure water (Milli Q, Millipore, USA) and the following chemicals were used: 96% H_2SO_4 (Merck, suprapur), 65% HNO_3 (Merck, suprapur), 30% HCl (Merck, suprapur), 30% H_2O_2 (Merck, p.a.), NaOH (Merck, p.a.), $NaBH_4$ (Fluka, purum p.a.), V_2O_5 (Merck, p.a.), Na_2SeO_3 (Sigma, 98%), selenomethioneine (SeMet, Sigma), selenocystine (SeCyst, Sigma).

Solution of V_2O_5 in H_2SO_4: 3.4 g V_2O_5 was added to 30 mL of Milli-Q water and slowly mixed with 170 mL of concentrated H_2SO_4 at 4 °C. The solution was kept in darkness.

1.2% $NaBH_4$ in 0.1 mol/L NaOH: 6 g $NaBH_4$ and 2 g NaOH were dissolved in 500 mL of Milli-Q water, filtered through a 0.45 μm filter and stored at 4 °C before use. The solution was prepared daily.

SeMet: 0.2412 mg Se/g stock solution of SeMet was prepared by dissolving 0.0598 g of SeMet in Milli-Q water.

SeCyst: 0.1985 mg Se/g stock solution of SeCyst was prepared by dissolving 0.0210 g of SeCyst in Milli-Q water.

Se(IV): stock solution was prepared by dissolving 0.2294 g Na_2SeO_3 in 100.00 g 0.1 mol/L HCl and stored at 4 °C.

The mass fractions of Se in stock solutions were checked by k_o-INAA.[10] Solutions with lower mass fractions of Se were prepared daily by dilution of stock solution.

b) *Sample pretreatment of blood/plasma samples:* 0.15 g of sample (blood, plasma) was weighed into a teflon tube (50 mL, Savillex). 1 mL 96% H_2SO_4 and 1 mL 30% H_2O_2 were added and the tube was heated in an aluminium block at 115 °C for 15 min. After cooling 2 mL 30% H_2O_2 was added and the tube was heated for 75 min at 115 °C. The solution was then cooled to room temperature, 100 μL solution of V_2O_5 in H_2SO_4 was added and well mixed. Heating for 20 min at 115 °C followed and the solution became blue coloured. To reduce selenium to Se(IV) which was necessary for hydride generation, 2 mL of concentrated HCl was added to the solution and heated for 10 min at 100 °C. Finally, the solution was diluted to 15 g with Milli-Q water and selenium was measured by the HG-AFS system. In the same way and with the same amount of chemicals as used for samples, a process blank was run every time. In the case of standard addition, a solution of Se(IV) was added to samples before digestion. Calibration solutions prepared in the same acid media as samples, samples and blank were measured at least twice. It is important to emphasize that the entire process of sample treatment from weighing to measuring was made in the same Teflon tube**.**

c) Sample pretreatment of protein fractions: 1 g of protein fraction was weighed into a teflon tube (25 mL, Savillex), 0.5 mL concentrated HNO_3 was added and the closed tube was heated for 10 min on a hot plate. Then 0.5 mL of 30% H_2O_2 was added three times and evaporated every time to ¼ volume. Reduction of selenium was made by the addition of 0.5 mL concentrated HCl and heating for 10 min on a hot plate. The solution was finally diluted to 2.5 g and measured by the HG-AFS system. In the same way and with the same amount of chemicals as for samples, a process blank was run every time. Calibration solutions prepared in the same acid media as samples, samples and blank were measured at least twice. And as was mentioned before it is important that the entire process of sample treatment from weighing to measuring was made in the same Teflon tube.

d) Measurement by HG-AFS: The continuous flow approach of an HG-AFS system was used to merge samples with reagents. The flow of carrier (0.05 mol/L HCl, 1 mL/min) or a sample solution was mixed in a PEEK (polyetheretherketone) cross connector with HCl (2 mol/L, 8 mL/min) and $NaBH_4$ (1.2%(m/V) in 0.1mol/L NaOH, 3 mL/min). Flow rates were adjusted at the peristaltic pump (Ismatec, MCP 380) by the choice of tubing

size (Tygon, LFL). The hydrogen selenide (H_2Se) and hydrogen which resulted from mixing in the cross piece were separated from liquid in a gas-liquid separator, swept from it with argon (260 mL/min) and dried in a Permapure dryer (Perma Pure Products). The dryer gas was nitrogen (3 L/min). H_2Se was atomized in an hydrogen/air flame in an atomic fluorescence spectrometer (model Excalibur, PS Analytical) with a Photron Se Super lamp.

Results and discussion

Determination of Se in blood/plasma samples

Optimisation of measurement by HG-AFS was first made and involved several variables. The ranges studied and optimum values found are shown in Table 1.

Table 1. Optimisation of HG-AFS system.

Variable	Range studied	Optimal value
Argon flow rate (mL/min)	100 - 375	260
Nitrogen flow rate (L/min)	2 - 3	3
Mass fraction of $NaBH_4$ (% m/V)	1.2 - 1.8	1.2
Conc. of HCl for hydride generation (mol/L)	2 - 4	2
Conc. of HCl in carrier (mol/L)	0 - 0.5	0.05

For Se determination in blood samples the procedure published by Tiran et al[9] was slightly modified. We altered the mass of sample, temperature of digestion, time of reduction of Se(VI) to Se (IV) and dilution mass. The mass of sample should be around 150 mg. At lower masses homogeneity can be a problem, while at higher masses we obtained too low and irreproducible results because of incomplete digestion, since there was not enough oxidant in solution. But if more sulphuric acid was added, problems with the density of liquid flow in continuous HG-AFS increased. The efficiency of digestion was improved by increasing the temperature from 100 to 115 ºC. The time of reduction was shortened from 20 to 10 min since experiments with radiotracer ^{75}Se showed that reduction is complete in this time.[11] Because of the great sensitivity of the detection system used (HG-AFS), the mass of solution to which the sample was diluted, was increased from 10 to 15 g. In this way the influence of sulphuric acid decreased.

As it is known that in hydride generation several interferences, including H_2SO_4 and HNO_3 can be present, the standard addition technique and the reagent matched calibration curve were used. A comparison between the results obtained by these two approaches was made. The results in Table 2 shows only slight differences. According to the t-test ($t_{exp} < t_{theor}$ for three of the four samples with 95% reliability), and because of its suitability for routine work, the calibration curve approach was chosen for further work.

Accuracy was tested by comparison of experimental results with recommended values for reference materials available. Recommended values for selenium in the two materials Seronorm Trace Elements Serum and Seronorm Trace Elements Whole Blood[12] are based on analytical data obtained in two highly skilled trace element laboratories by ET-AAS and are the arithmetic means of all values obtained. These reference materials are stable and lyophilized materials of human origin and should be reconstituted before use. So they are especially suitable for control since the matrix is nearly the same as in samples. Accuracy was also checked by analysing internal standards in which selenium was determined by RNAA.[13,14] The results of comparison are shown in Table 2 and are in good agreement. The average recovery in determination of Se in Seronorm Trace Elements Serum as determined by standard addition was 0.98 ± 0.08 (n=48). In routine work results were not corrected for recovery.

Linearity for an aqueous solution was up to 200 ng/g and 13 ng/g in a blood matrix solution. R^2 of the linear regression analysis based on the method of least squares was always higher than 0.996.

The detection limit (DL) was determined by repeated analysis of blanks involving the entire process, decomposition included, and was calculated as the three-fold multiple of the standard deviation of the blank divided by the slope. This DL was 0.2 ng/g of measuring solution (n=15) or 20 ng/g for a 0.15 g sample, dilution to 15 g of measuring solution and continuous flow HG-AFS. Prohaska et al[15] obtained 0.5 ng/mL by HG-ICP-AES, 0.7 ng/mL by ET-AAS and 0.05 ng/mL by FI-ET-AAS, all based on the measuring solution.

The repeatability of the determination of selenium in Seronorm Trace Elements Serum was under 7% (RSD_r) and the reproducibility over a period of 6 months was under 10% (RSD_R) calculated by ANOVA. The repeatability, reproducibility and

Table 2. Comparison of results obtained by the reagent matched calibration curve approach and the standard addition technique by HG-AFS with recommended values and with results obtained by RNAA.

Sample	Mass fraction (ng Se/g)			
	HG-AFS[a]		RNAA	Recommended value (Range of given values)
	Calibration curve	Standard addition		
Seronorm Trace Elements Serum	79 ± 6 (36)	79 ± 9 (6)		80 (70-92)
Plasma 1[b]	61 ± 2 (6)	70 ± 3 (6)		
Whole blood 1[b]	63 ± 4 (6)	66 ± 4 (6)		
Whole blood 2[b]	67± 9 (16)	67 ± 9 (6)	66; 58	
Whole blood 3[c]	82 ± 4 (4)		82; 68	
Seronorm Trace Elements Whole Blood	80 ± 4 (12)			78 (76-87)

[a] average ± standard deviation (number of determinations).
[b] pooled sample of 5–7 persons.
[c] individual blood sample.

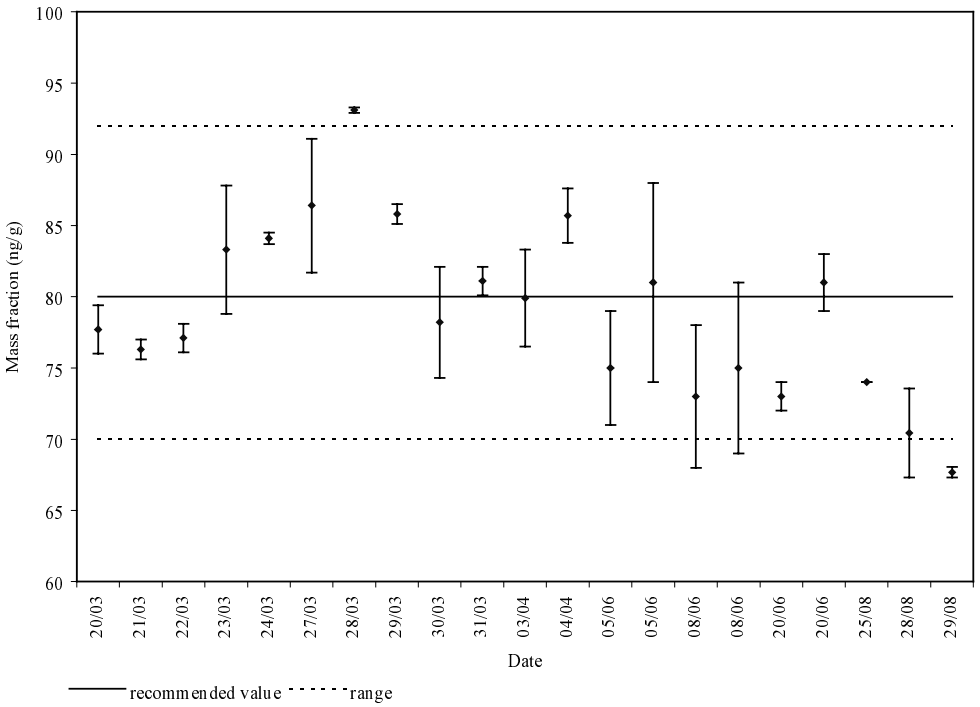

Figure 1. Quality control chart for determination of Se in Seronorm Trace Elements Serum (recommended value 80 ng/g, range of determinations 70-92 ng/g).

accuracy of the procedure can also be checked with a quality control chart. As is seen in Figure 1, all results lie around the recommended value within the range of determinations of the reference laboratories for Seronorm Trace Elements Serum.

A total uncertainty budget performed[16] for the determination of Se in Seronorm Trace Elements Serum showed that the major sources of uncertainty were the uncertainty of the recovery of the procedure, the measurement of peak heights and the purity of Na_2SeO_3. The measurement uncertainty expressed relatively was 6%.

The procedure developed was used to determine selenium in real samples. An average mass fraction of selenium in 43 samples of whole blood of residents of Slovenia (21-52 year old subjects) was 87 ± 13 ng/g, with a median of 86 ng/g and a range of 65-130 ng/g. Byrne and Klemenc[17] obtained by activation analyses similar value of 86 ± 26 ng/mL for 26 subjects from Slovenia. These values fall within the range of literature data for the general population of 58-234 ng/g[18] and in the range of data for some European countries of 34-166 ng/mL.[15,17,19]

Determination of Se in protein fractions

SeMet which is among the most acid-resistant selenium compounds found in biological fluids,[20] was used during the development and optimization of the digestion procedure of protein fractions because there was no appropriate reference material available for the determination of digestion efficiency. Some parameters of the procedure published in the literature[21] were modified. The ratio between the mass of solution and volume of HNO_3, the time of heating, the presence of H_2O_2 and the influence of the duration of evaporation after the addition of H_2O_2 were studied (Table 3).

SeMet with a mass fraction between 20 and 200 ng/g was completely digested under optimal conditions. The average recovery of digestion of SeMet with a mass fraction of 200 ng/g was 91 ± 5% (n =15). The same procedure was used for selenocystine with the same mass fraction and the average recovery was 91 ± 3%. Correction for recovery was applied in calculation of results.

The detection limit was determined by repeated analysis of blanks involving the entire process and was 0.2 ng/g of measuring solution (n=15) or 0.5 ng/g sample for a 1 g sample and dilution to 2.5 g of measuring solution. Because of its low detection limit this procedure was useful for determination of Se in SeP.

Table 3. Optimization of digestion of protein fractions.

Variable	Range studied	Optimal value
Ratio mass of sample solution (g): volume of HNO_3 (mL)	2:0 - 2:3	1: 0.5
Time of heating (min)	0 - 30	10
Volume of H_2O_2 (mL)	0-1.5	1.5
Evaporization after addition H_2O_2 (min)	0-15	15*

* evaporation for 15 min or expressed differently, to ¼ of volume of digested solution after each addition of H_2O_2.

The repeatability of the determination of selenium in SeMet was between 1-8% (RSD_r) and the reproducibility over a period of 6 months was under 6% (RSD_R) calculated by ANOVA.

Isolation of SeP from plasma by affinity chromatography

The slightly modified procedure of Harrison et al[5], described in details in the Experimental section, was used to separate plasma proteins. GSH-Px + Alb (fraction A) was eluted from the affinity column with equilibrium buffer. SeP (fraction B) bound to heparin-sepharose was then eluted with elution buffer and collected in subfractions. Retention of Se on the column packing was checked with Se determination by RNAA[13,14] in pure heparin-sepharose and in heparin-sepharose after several separations of real samples. 7% of the total selenium was always retained on the column, independently of the number of separations made on the same column. The average recovery of separation, the ratio between Se applied to the column and Se obtained from the column, was 92 ± 15% (n= 5). The distribution of selenium within SeP was similar for all samples, and for four of them is presented in Figure 2.

The curves differed only in the height of the peak due to the differences in selenium mass fraction in the various samples. Following this distribution pattern in further work it would be possible to pool fractions from 7.5 mL to 35 mL and lyophilize them to reduce dilution. Hence the result for percentage of Se in SeP in plasma would be obtained in just two steps: Se determination in fraction A and in fraction B. In this way the procedure would be faster and more convenient for routine determination of the Se status of plasma SeP.

The procedure was also tested regarding the possible absorption of Se on the test tube walls and the influence of lyophilisation on the Se mass fraction. Fractions were

stored at –21 °C prior to Se determination. Absorption of selenium on the walls of test tubes of fraction A and B after 14 days of storage was observed, up to 28%. This effect was minimized by the addition of 200 μL of 65% HNO_3 (Table 4). The reproducibility of determination of Se in protein subfractions after the addition of HNO_3 is shown in Table 5.

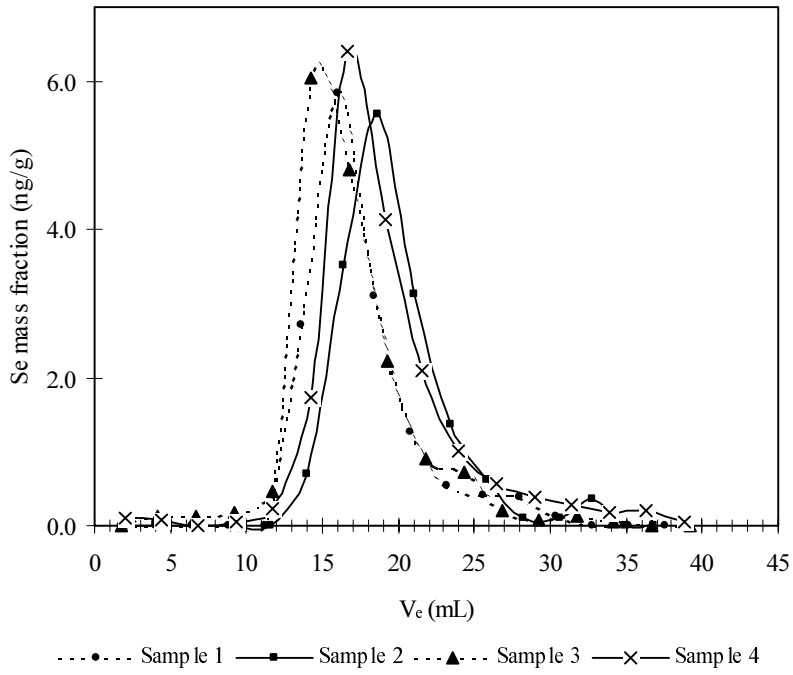

Figure 2. Distribution of Se in SeP after affinity chromatography for four different samples.

The influence of lyophilisation was tested by a comparison between results obtained for lyophilised and non-lyophilised fraction A. The results showed that during lyophilisation no loss of Se occurred (Table 6). This is a confirmation of the statement of Heydorn who wrote that lyophilisation is suitable for removal of water from biological samples (blood, plasma) without significant loss of trace amounts of Se.[22]

As a result of our tests it was found that the procedure for determination of the distribution of plasma Se between GSH-Px, Alb and SeP could be shortened from the numerous analyses (about 40) by Harrison et al[5], to only 3 analyses.

This method was used on 5 plasma samples (25 – 41 years old subjects). The average value of Se in SeP was 70 ± 9% and the percentage of Se in GSH-Px + Alb was 30 ± 9%. After that we used the method on a larger number of samples from Idrija residents living in the mercury contaminated environment, and in some cases

additionally exposed to physical stress or metal chelator (DIMAVAL) treatment.[23] The average value of Se in SeP in the plasma of Idrija residents, without "special cases", was 70 ± 5% (n=17).[23] The results of both of our studies, reference No.23 and this work, were also comparable with the value found by Plecko, 68 ± 7%(n=17) Se in SeP for the general population[24], while Harrison et al[5] found a somewhat lower value of 53 ± 6% (n=21). Plecko[24] and Harrison[5] used separation on a heparin sepharose column and ET-AAS detection, while Bendahl used extraction on a solid phase and ICP-MS detection and obtained results in the range 27-50% (n=5).[25] The differences between the results could be due to the small number of samples in all studies and to the use of different methods with different detection limits (HG-AFS 0.5 ng/g, ICP-MS 2 ng/g, ET-AAS 0.8 ng/g).[5,25]

Table 4. Comparison of Se masses in fraction B (sum of analysed subfractions) stored with and without the addition of 200 μL of 65% HNO_3 measured after a 14 day interval.

Sample (n)	HNO_3	Se in SeP (ng)		% of change*
		1.determination	2.determination	
1 (14)		110	83	- 25
2 (8)		83	62	- 25
3 (14)	−	64	50	- 22
4 (14)		82	60	- 28
Average				**- 25**
5 (7)		84	77	- 8
6 (7)	+	42	43	+ 2
7 (4)		29	31	+ 7
8 (4)		46	46	0
Average				**0**

* % of increase or decrease of selenium mass between two measurements carried out after a 14 day interval. n - number of analysed subfractions.

Table 5. Time reproducibility of determination of Se in protein subfractions after the addition of HNO_3.

Subfraction No.	Mass fraction (ng Se/g)		Time between determinations
	1. determination	2. determination	
6	0.5	0.5	6 days
7	5.9	6.4	6 days
7	5.7	5.8	3 months
8	5.5	5.6	3 months

Table 6. Influence of lyophilisation on Se content in fraction A.

Fraction A	Mass of Se* (ng)	
	Non-lyophilised	Lyophilised
1	63, 58	65 ± 6 (3)
2	63 ± 5 (3)	63, 64

* average ± standard deviation (number of determinations).

Conclusions

With a digestion procedure using H_2SO_4, H_2O_2 and V_2O_5 in H_2SO_4 solution a detection limit of 20 ng Se/g of blood sample was achieved, while for protein chromatographic fractions digestion with HNO_3 and H_2O_2 gave 0.5 ng Se/g of fraction, both digestions coupled with HG-AFS detection. Compared to other methods found in the literature, our procedures were simple, used only a small mass of sample, low temperatures and less dangerous chemicals for digestion, and whole procedure from weighing to measuring was carried out in the same teflon tubes.

In the chromatographic separation we found that heparin-sepharose used for isolation of SeP could be used for several separations because retention of Se on the column is constant, 7% of total Se, and independent of the number of separations on the same column. Lyophilisation of pooled chromatographic protein fractions could efficiently reduce the number of analyses needed for determination of Se in SeP in plasma. Absorption of Se on the test tube walls should be minimized by the addition of HNO_3 to the fractions.

Using procedures incorporating these improvements, the selenium mass fraction and the distribution of selenium between SeP and other plasma proteins (GSH-Px + Alb) in real blood samples were analysed and the results were comparable with the literature data.

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Povzetek

Za določanje selena v vzorcih krvi in plazme smo razvili postopek, ki povezuje razkroj s H_2SO_4, H_2O_2 in V_2O_5 v H_2SO_4 in HG-AFS detekcijski sistem. Za določanje Se v beljakovinskih frakcijah plazme pa je bil dovolj učinkovit razkroj s HNO_3 in H_2O_2, ki ravno tako kot prvi v povezavi s HG-AFS daje zanesljive rezultate. Po afinitetni kromatografiji potrebni za izolacijo plazemskih beljakovin, smo subfrakcije frakcije GSH-Px+Alb združili, liofilizirali in tako zmanjšali število določitev iz 16 na eno. Glavni prednosti postopkov sta nizka meja zaznavnosti (0.2 ng Se/g raztopine) in možnost analize velikega števila vzorcev v kratkem časovnem obdobju.

Z razvitimi postopki smo določili Se v realnih vzorcih. Povprečen masni delež Se v krvi prebivalcev Slovenije je bil 87 ± 13 ng/g (n=43), delež Se vezanega v SeP v plazmi pa je bil 70 ± 9% (n=5). Oba podatka se ujemata s podatki, ki jih navaja literatura za splošno populacijo.