SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
15 (2009)

DIRECT DETERMINATION OF SELENIUM IN HUMAN URINE BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY WITH DEUTERIUM BACKGROUND CORRECTION

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Received September 30, 2009

The elaboration of methodology for the direct determination of Se in urine by electrothermal atomic absorption spectrometry (ETAAS) has been contributed by several authors with the predominant use of Zeeman-background correction systems which is less affected by spectral effects. A systematic study of several recommended (Pd (nitrate), Pd + Mg), less commonly applied (Pd + Sr (nitrate), Pd + Mg + Sr (nitrate)) and new chemical modifiers (Pd + Sr (iodide), Pd + Ag, Pd + Ag + Sr (iodide), Pd + Mg + Sr (iodide)) resulted in the optimization of atomization conditions under which accurate analysis became possible also with the use of deuterium lamp background correction. Pd (nitrate) + Sr (iodide) + Ag (nitrate) was found to be preferable for the determination of Se in urine. The use of this modifier resulted in the lowest detection limit and characteristic mass value of 3.6 μg l^{-1} of Se (10 μl aliquots of dispensed urine) and 17 pg, respectively and

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ensured the analytical recoveries close to 100 %. Applying the modifier to standards and samples has enabled the use of matrix-free standard solutions for attaining accurate analysis of diluted urine samples. The accuracy of the analysis was verified by the use of commercially available quality control reference materials and also by comparison with radiochemical neutron activation analysis (RNAA) method.

Introduction

Accurate determination of Se in urine is important since urinary Se concentration directly reflects uptake and total content of this essentially and toxicologically important element [1]. ETAAS method seems to be very useful for this purpose since its sensitivity is adequate enough for determination of Se even at levels of general and unexposed population. However, this determination is not easy and both spectral and non-spectral interferences are effective at the direct determination of Se [2-5], the spectral interferences being more severe when deuterium lamp (D_2) was used, if compared to Zeeman background correction methods [2]. Nevertheless, the determination can be difficult even using instrumentation equipped with zeeman-effect background correction [3]. Interferences from PO and NO molecular radicals produced during the decomposition of phosphates and urea were found to be the most responsible for these problems [5]. To overcome these interferences several separation/ preconcentration methods can be used [6]; however, these methods are time consuming and less appropriate for routine analysis. The most successful approach to decrease or to eliminate interference effects in consideration has been the introduction of stabilized temperature platform furnace concept (STPF), which has often been coupled with the use of chemical modifiers [7]. Nickel alone [8-10] or combined with Pd and NH_4NO_3 [11] was widely used for overcoming problems occurring during the determination of Se in urine. The use of Ni may be more useful for reduction of molecular absorption of PO in comparison with Pd chemical modifier. In addition there are no problems with possible spectral interference due to the nearby spectral lines of Pd and Se [3]. Tsalev *et al.* [12] recommended the use of Rh as chemical modifier for overcoming interferences of phosphates during the determination of Se in biological samples; the interference may also be overcome by the use of ammonium molybdate; however, Pd with Mg must be added for analyte stabilization [13]. Drake and Hain [14] applied Pd with Mg and Ba mixed chemical modifier for the determination of urinary Se using instrumentation equipped with D_2 background correction (BC); however, interference could not be overcome and overcorrection occurred. Covering of graphite furnace with Zr and Rh as permanent modifiers may significantly

decrease the spectral effects of urine matrix onto Se determination when instrumentation with D_2BC was used whether urine samples are diluted in 1:1 ratio with the solution containing 1% (v/v) HNO_3 and 0.02 % (v/v) cetyltrimethylammonium chloride and Rh is additionally injected into the cuvette together with a sample [15]. However, this procedure does not eliminate non spectral effects.

Regarding the determination of urinary Se, the composition of chemical modifier cannot be adopted only with the aim to correct spectral effects. High volatility and different stability of urinary Se species (selenite (Se(IV)) and trimethylselenonium (Se(-II)) ($TMSe^+$) in the presence of various chemical modifiers is another problem which must be taken into consideration [4,16-20]. In order to overcome these problems, several authors [4, 16-20] investigated influence of different chemical modifiers such nitrates of Pd, Ni, Mg, Ca, La, Eu [16], Sb, Cd, Mn, Mo, Ni, KI, KIO_3, Ag, Th, Tl, Zn and Zr [4], Ni, Mg, Cu, Cu + Mg, Pd, Pd + Mg [17], Pd and Ni [18,20], Ni, Cu, Pd [19] on various Se species. Dočekalová *et al.* [16] recommended the use of Pd+Mg chemical modifier for determination of Se in body fluids under conditions where molar excess of Pd and Mg of 300 and 30000x over Se was used. On the other hand, Johannessen *et al.* [17] reported that although Pd and Pd+Mg are efficient for stabilization of selenite and selenate (Se(VI)), significant decrease in sensitivity for $TMSe^+$ of about 55 % and 85 % occurred in the presence of Pd alone or Pd+Mg chemical modifier. Both chemical modifiers thus are not useful for the determination of urinary Se since the error of the determination will depend on concentration of $TMSe^+$ which may vary from 10 % for general population to 50-60 % for population exposed to heavy metals as $TMSe^+$ plays and important role during organism detoxication [21]. According to Laborda *et al.* [18] the ability of Pd for $TMSe^+$ stabilization depends on the fact whether Pd is present in reducible or irreducible form. While in the presence of irreducible palladium (PdO) both urinary selenium compounds (selenite and $TMSe^+$) are stabilized to the same extent, in the presence of the later one a decrease in sensitivity of about 50 % will occur for $TMSe^+$ (urea in urine acts as reducing agent). These problems were not observed for Ni [18], although in its presence significant decrease of pyrolysis temperature was observed in urine matrix in contrast to matrix-free conditions and analytical recoveries of about 70-90 % were found and even additional use of Mg (nitrate) did not remove this problem. Campillo *et al.* [20] reported that Pd in comparison with Ni is more useful for stabilization of organically and inorganically bound selenium when urine samples are injected into the cuvette together with a mixture containing 0.65 % (w/v) HNO_3 + 15 % (w/v) H_2O_2. Alexander, Saeed and Thomassen [4] reported that Mo, Ni a Ag stabilize effectively organically bound selenium in urine up to the temperatures of 900, 1100 and 1300 °C and thus they are strongly recommended for this purpose.

Similarly, Ir permanent chemical modifier was recommended for urinary Se determination; however, in order to achieve analytical recoveries close to 100 % in samples containing high percentage of $TMSe^+$, microwave digestion of the samples or *in situ* oxidation of the sample in the cuvette with mixture containing 5% (w/v) $KMnO_4$, 63% (w/w) HNO_3 and 30% (w/w) H_2O_2 must be performed [22].

It is clearly evident that direct determination of Se in urine is still a great challenge owing to all of the above-mentioned problems. In order to develop direct, quick and accurate method for determination of Se in urine by means of ETAAS with D_2 background correction which is still the most widely used method in analytical laboratories, a critical comparative study of different chemical modifiers was undertaken.

Experimental

Instrumentation

Avanta P double beam atomic absorption spectrometer (GBC Scientific Equipment Pty Ltd., Australia) equipped with GF 3000 graphite furnace, auto sampler PAL 3000 and deuterium arc background corrector were used. Super lamp (Photron Pty. Ltd., Australia) was the line source (lamp current 13 mA, wavelength 196 nm, and spectral band pass 2 nm). Both peak height and peak area absorbance values were measured. Pyrolytically coated graphite tubes (Schunk, Germany, cat. no. 56GB715) with a preinstalled pyrolytic graphite L'vov platform were used. Argon was used as sheat gas; the internal gas flow in the graphite tube was interrupted during the atomization step.

Reagents and Standards

Reagents of analytical grade or higher quality were used. All the solutions were prepared using deionized water. Water used in all experiments was purified using the UltraClear (SG, Germany) pure water system to 0.05 μS cm^{-1}. All glass and plastic ware were immersed in 2 M nitric acid for 24 hours followed by rinsing with deionized water.

Selenium solution (Se(IV)) of 1 g l^{-1} Se was obtained from Merck (Darmstadt, Germany). Nitric acid (65 % m/m) was Suprapur® (Analytika Ltd., Prague, the Czech Republic). Palladium solution of 10 g l^{-1} Pd in 10 % v/v HNO_3 was obtained from CPI International (USA). The solution of 1g l^{-1} of Pd was prepared by diluting an appropriate amount of a 10 g l^{-1} with water. Platinum (H_2PtCl_6 in 2 mol l^{-1} HCl) solution of 1 g l^{-1} Pt was obtained from Fluka (Buchs, Switzerland).

Phosphorus solution (1 g l^{-1}) was prepared by dissolving of ammonium dihydrogen phosphate (Analytika) in water. Magnesium nitrate and silver nitrate of 10 g l^{-1} Mg or Ag and strontium nitrate and nickel nitrate solution of 1g l^{-1} Sr and Ni, respectively, were prepared by dissolving the appropriate amounts of the salts (Lachema, Brno, the Czech Republic) in water.

Procedure

Sample Preparation

First morning urine samples were collected into polyethylene vessels and stored at the temperature of 4 °C until they were analyzed. Prior to the analysis, samples were diluted in 1+3 ratio with water.

The commercially supplied urine quality control materials: Lyphochek Lot No. 69011 (BIO-RAD, USA) and Seronorm™ 2525 (Sero, Norway) were reconstituted according to the instructions given in the certificates.

Analytical Procedure

For optimization studies, real urine samples diluted to 1+3 with water and spiked with 50 μg l^{-1} of Se were used. A 10 μl aliquot of this solution and appropriate volumes of modifiers were injected together on the platform by means of auto sampler. To achieve a total mass of the following investigated chemical modifiers: (i) 3 μg Pd, 5 μg Ni, 5 μg Pt, (ii) 10-50 μg Pd, (iii) 30 μg Pd+(1-100) μg Mg, (iv) 30 μg Pd+(1-10) μg Sr, (v) 30 μg Pd+(5-30) μg Ag, (vi) 30 μg Pd+(1-5) μg Mg+(1-5) Sr and (vii) 30 μg Pd+(2-10) μg Ag+(1-5) μg Sr, the following injection volumes were taken: (i) 3 μl 1g l^{-1} Pd, 5 μl 1g l^{-1} Ni, or Pt, (ii) 1-5 μl 10 g l^{-1} Pd, (iii) 3 μl 10 g l^{-1} Pd+(1-10) μl 1 or 10 g l^{-1} Mg, (iv) 3 μl 10 g l^{-1} Pd+(1-10) μl 1 g l^{-1} Sr, (v) 3 μl 10 g l^{-1} Pd+(1-6) μl 5 g l^{-1} Ag, (vi) 3 μl 10 g l^{-1} Pd+(1-5) μl 1 g l^{-1} Mg +(1-5) μl 1 g l^{-1} Sr and (vii) 3 μl 10 g l^{-1} Pd+(2-10) μl 1 g l^{-1}Ag+(1-5) μl 1 g l^{-1} Sr. The temperature program used throughout this work is presented in Table 1. Five aqueous standards were used to obtain the calibration curves and two standard additions were also made. The concentrations of solutions used in the calibration ranged from 10 μg l^{-1} to 100 μg l^{-1} of Se. The calibration curves were linear in the concentration ranges of these standards. The direct calibration and standard addition method were controlled and compared by means of the instrument software. The correlation coefficients found were at least 0.999 for direct calibration and 0.99 for standard additions.

Table I Electrothermal program for determination of Se in urine using an atomic absorption spectrometer GBC Avanta P

	Injection	Drying		Pyrolysis		Atomization	Cleaning	Cooling
Temperature, °C	65	120	280	[a]	[a]	[b]	2600	40
Ramp, s	5	20	15	5	0		1	25
Hold, s	10	15	5	15	1	2	1	5
Ar flow, l min^{-1}	3	3	3	3	0	0	3	3

[a] The pyrolysis and atomization temperatures depended on the type of a chemical modifier used.
[b] 2000 °C s^{-1}

Results and Discussion

Comparison of Chemical Modifiers for Elimination of Phosphate Interference

As it was mentioned in the introduction section, phosphates represent the most serious source of interferences occurring during the direct determination of urinary Se. It can be seen from Fig. 1 that the amount as low as 0.5 μg P decreases the integrated absorbance of Se by about 50 %. This interference can be kept under good control in the presence of Pd chemical modifier. When 5 μg Pd (nitrate) was used, up to 10 μg P in the form of ammonium phosphate could be tolerated without changes of sensitivity. The presence of Pt (H_2PtCl_6) chemical modifier can also overcome the interference from phosphate matrix; however, the lower sensitivity in comparison to those obtained for Pd modifier can be seen. On the other hand, the presence of the most widely used Ni chemical modifier did not lead to elimination of this interference and in the presence of 5 μg Ni nitrate significant decrease of integrated absorbance of about 50 % has occurred in the presence of only 1 μg P. These observations correspond well to those reported previously by several authors [18] that significantly high amounts of this modifier must be applied during the real sample analysis. However, this leads to unwanted contamination of atomizer and makes the later analysis of this element very difficult.

Comparison of Chemical Modifiers for Elimination of Sulphate Interference

In addition to interference from phosphate matrix, determination of Se is very sensitive to the presence of sulphates. Therefore, this interference has to be kept in mind and controlled regularly during the analysis of urine. As it can be seen from Fig. 2, the presence of 2 μg S results in a decrease in sensitivity of about 50 % at conditions when no chemical modifier was used. As expected, this interference can be overcome in the presence of Pd + Sr modifier mixture.

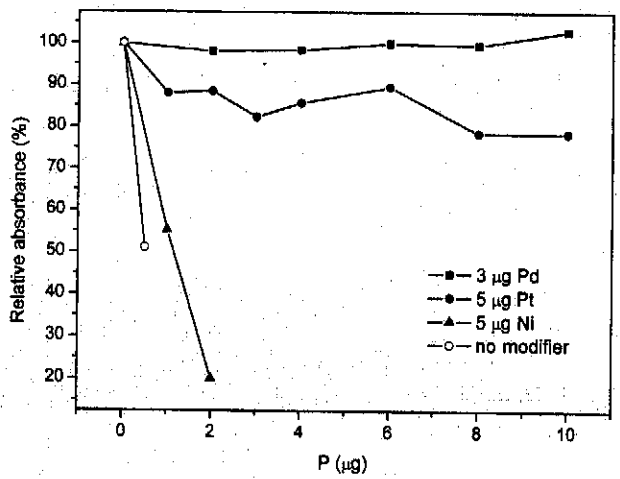

Fig. 1 Influence of P amount on the relative absorbance signal of Se in the absence and presence of selected modifiers. The relative absorbance is defined as: Relative absorbance = integrated absorbance of 500 pg Se in presence of P within the investigated range/ integrated absorbance of 500 pg Se

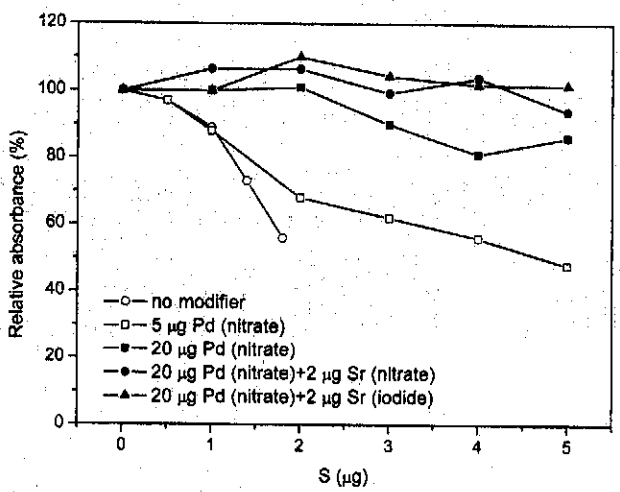

Fig. 2 Influence of S amount on the relative absorbance signal of Se in the absence and presence of selected modifiers. The relative absorbance is defined as: Relative absorbance = integrated absorbance of 500 pg Se in presence of S within the investigated range/ integrated absorbance of 500 pg Se

Palladium alone is also efficient for elimination of this interference; however, sufficiently high amount of a chemical modifier must be used for this purpose. While in the presence of 5 µg Pd significant decrease in integrated absorbance was

observed already for 0.5 μg S, in the presence of 20 μg Pd up to 2 μg S in the form of sodium sulphate could be tolerated. It may be expected that this fact is the reason for conclusions of several authors [23] that Pd is not efficient to overcome sulphate interference. In the presence of sulphates poisoning of modifier may play an important role resulting in a significant decrease in number of reactive centers necessary for selenium binding that lead to limited analyte stabilization resulting in a decrease in sensitivity. As regards the overcoming of the sulphate interference, the best results were achieved in the presence of mixed Pd + Sr modifier. Although for this purpose Sr in the form of nitrate was recommended previously [24], according to our observations the use of Sr as iodide may be more useful. As it is demonstrated in Fig. 2, both chemical modifiers have similar efficiency to overcome this interference; however, when Sr iodide is used, significantly lower background absorbances are observed, and that may play an important role during the analysis of urine.

Testing Modifiers Mixtures and Optimization of Amount of Chemical Modifiers

For the purpose of direct determination of urinary Se the following chemical modifier mixtures have been tested: Pd (nitrate), Pd (nitrate) + Mg (nitrate), Pd (nitrate) + Sr (nitrate), Pd (nitrate) + Sr (iodide), Pd (nitrate) + Ag (nitrate), Pd (nitrate) + Sr (iodide) + Mg (nitrate), Pd (nitrate) + Sr (iodide) + Ag (nitrate). With the exception of Pd and Pd + Mg, none of these chemical modifiers was previously used and applied for determination of urinary Se; moreover, chemical modifiers like Pd (nitrate) + Sr (iodide), Pd + Mg + Sr (iodide), Pd + Ag, Pd + Ag + Sr (iodide) have not been described in analytical practice to this date. In the all cases, Pd was the main component of these chemical modifier mixtures due to its high efficiency to overcome the interference from urine matrix.

Influence of Pd chemical modifier within the range of 5-50 μg on the specific and nonspecific absorbance signal was studied. It was found that if modifier amounts lower than 20 μg were used, an overcorrection occurred; however, as low amount as 30 μg Pd resulted in maximum decrease in background absorbance and maximum increase in sensitivity. Finally, 30 μg Pd was chosen as the optimum amount for determination in urine and this was used during optimization of other modifier compounds. Relating the use of Pd + Mg chemical modifier, the amount of Mg was optimized within the range of 1-100 μg. The amount of 2 μg was chosen since higher masses led to an increase in background absorbance and produced neither additional signal stabilization nor increase in sensitivity.

According to several authors [4] Ag may be used as an efficient chemical modifier during the determination of Se in urine due to its ability to stabilize both

organically and inorganically bound Se and to overcome the interference from urine matrix. However, we have found that the problem with overcorrection will occur in the presence of only Ag and even such high amounts of Ag as 100 μg cannot overcome this problem. Due to this fact, we have investigated the addition 5-30 μg of Ag to Pd modifier. It was found that within the investigated range the addition of silver (nitrate) did not lead to a generation of high background absorbance and silver addition increased Se stabilization. For this purpose 10 μg Ag was found to be optimal. Except of Ag addition, Sr was additionally tested in a mixture with Pd. Strontium was applied as both nitrate and iodide within the range of 1-5 μg Sr; finally 2 μg were found to be optimal. In the presence of higher amounts, a significant increase in background values was observed and no additional signal delaying has occurred. It was also found that in the presence of Pd + Sr (iodide) significantly lower background absorbances were measured in comparison with previously recommended Pd + Sr (nitrate) modifier. It was also found out, that Pd + Sr (iodide) is more efficient for analyte stabilization when compared with Pd+Sr (nitrate). While in the presence of $Pd(NO_3)_2$ + $Sr(NO_3)_2$ mixture in a graphite furnace Pd will be present mainly as PdO, in the presence of $Pd(NO_3)_2$ + SrI_2 mixture the reaction will take place to form PdI_2. It is expected that during the graphite furnace heating palladium iodide decomposes to form elementary Pd. Shift of atomization signal to higher temperatures may be thus attributed to a different mechanism of interaction between analyte and modifier. Since creation of black precipitate of PdI_2 takes place already at room temperature after mixing $Pd(NO_3)_2$ and SrI_2, the two chemical modifiers must be injected into the cuvette separately. Similar problems were observed also during an injection of SrI_2 together with $AgNO_3$.

Regarding the optimization of chemical modifiers containing three components, the following mixtures within the ranges shown in parentheses were tested in order to obtain the best analyte stabilization, sensitivity and the maximum decrease in background absorbance: 30 μg Pd + (1-5) μg Mg + (1-5) μg Sr and 30 μg Pd + (2-10) μg Ag + (1-5) μg Sr. Based on these criteria, the following amounts were finally selected to produce the best results: 30 μg Pd (nitrate) + 2 μg Sr (iodide), 30 μg Pd (nitrate) + 2 μg Mg (nitrate) + 2 μg Sr (iodide) and 30 μg Pd (nitrate) + 2 μg Ag (nitrate) + 2 μg Sr (iodide).

Pyrolysis and Atomization Curves

The effect of pyrolysis and atomization temperature on the specific and background absorption signal in the presence of all the chemical modifiers studied is shown in Fig. 3. It can be seen that using Pd as the chemical modifier, the optimum ashing and atomization temperatures correspond to 1200 °C and 2100 °C

(peak area measurement), respectively; however the addition of further component such Ag and Sr, results in additional increase in the pyrolysis and atomization temperatures (1300 and 2200 °C). The addition of Mg to Pd chemical modifier did not influence the thermal stabilization and affected negatively the background values. Typical background absorbances of real samples in the presence of all the chemical modifiers were bellow 0.12 A and could be easily compensated using deuterium corrector.

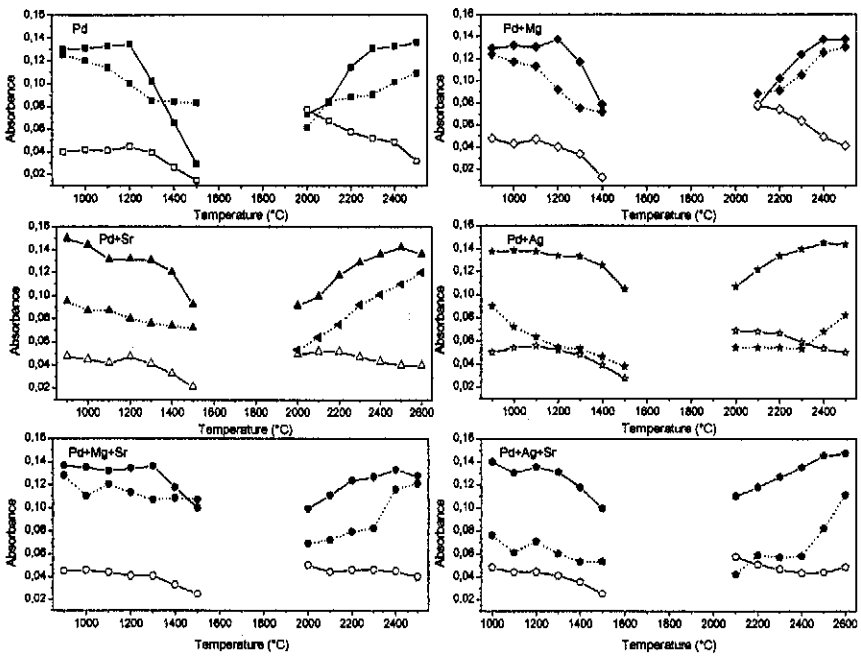

Fig. 3 Effect of pyrolysis and atomization temperatures on Se (solid lines) and background (dotted lines) absorption signals in urine diluted in 1:3 ratio and spiked with 50 μg l^{-1} of Se in the presence of 30 μg Pd, 30 μg Pd + 2 μg Mg, 30 μg Pd + 2 μg Sr, 30 μg Pd + 10 μg Ag, 30 μg Pd + 2 μg Mg + 2 μg Sr and 30 μg Pd + 2 μg Ag + 2 μg Sr based on measurement of peak-area (open symbols) and peak-height (solid symbols) absorbance

Evaluation of Matrix Effects and Analytical Recoveries Study

To check possible interferences from the whole urine matrix in the presence of all the investigated chemical modifiers, the slopes of analytical curves found with matrix-free aqueous standards ("simple calibration") and with that of standard addition were determined for both peak height and peak area measurements (See

Table II). Standard additions were applied to four urine samples (U1-U4). The pyrolysis and atomization temperatures used were, respectively, 1200 and 2100 °C for conditions with Pd alone or Pd with Mg chemical modifier and 1300 and 2200 °C for other investigated modifiers. The slope values are listed for simple calibration and slope differences are shown for the standard additions. It can be estimated from the data in Table II that the deviations of slopes caused by the matrix effects are not significantly different for both peak height and peak area measurements (p = 0.934, paired t-test). The results also show that Pd+Ag, Pd+Sr+Ag were the most efficient for the elimination of matrix effect. Using these modifiers, the maximum difference between the calibration and standard addition slopes (both for peak height and peak area) was about 15 %, thus a simple aqueous calibration can be used for Se measurement in the presence of both chemical modifiers.

The attention was focused also on the evaluation of analytical recoveries obtained for four different real urine samples spiked with 20 μg l^{-1} of Se in the presence of all investigated chemical modifiers (see Table III). It can be seen from Table III that the presence of Pd + Ag + Sr chemical modifier gave the best results. Although the values obtained in the presence of all the investigated chemical modifiers were better than 90 %, the lowest variability of results was observed in the presence of Pd + Ag + Sr modifier.

Table II Slopes of the calibration lines of Se (μg^{-1} l) and differences (%) between the slopes of standard additions method and slope of direct aqueous calibration in presence of all investigated chemical modifiers

	Peak height					Peak area				
	Slope	Difference, %				Slope	Difference, %			
Modifier[a]	Aqueous	U 1[b]	U 2	U 3	U 4	Aqueous	U 1	U 2	U 3	U 4
Pd	0.00239	–0.7	–18.4	–3.7	–8.7	0.00129	–8.2	–22.5	–14.4	6.1
Pd + Mg	0.00234	–15.4	13.6	1.1	–13.1	0.00154	–2.1	–1.6	7.2	–23.0
Pd + Sr	0.00240	13.2	10.7	10.8	–18.8	0.00119	1.6	–3.0	14.0	–9.4
Pd + Ag	0.00226	–15.0	–6.4	–11.0	–13.3	0.00136	–5.3	–3.1	0.9	1.9
Pd + Mg + Sr	0.00231	9.1	16.6	6.7	–3.1	0.00110	–8.7	0.1	9.0	8.7
Pd + Ag + Sr	0.00240	14.8	12.2	–3.2	3.2	0.00110	5.3	14.5	1.4	12.6

[a] 30 μg Pd, 30 μg Pd + 2 μg Mg, 30 μg Pd + 2 μg Sr, 30 μg Pd + 10 μg Ag, 30 μg Pd + 2 μg Mg + 2 μg Sr, 30 μg Pd + 5 μg Ag + 2 μg Sr; [b] U 1, U 2, U 3, U 4 real urine samples

Analytical Characteristics

Limits of detection (LOD) and limits of quantification (LOQ) based on repeated analysis of a sample with low Se concentration were calculated as $3S_{blk}/m$ and/or $10S_{blk}/m$, where S_{blk} means the standard deviation of 10 blank measurements and m is the slope of the calibration graph. Characteristic masses were calculated from the slopes of the standard additions technique using the equation $m_0 = (0.0044 \times V)$

Table III Analytical recoveries of Se determination in real urine obtained in presence of all chemical modifiers studied

Modifier	Peak height	Peak area
	Recovery [a,b] (%)	Recovery [a,b] (%)
30 μg Pd	92 ± 12	92 ± 10
30 μg Pd + 2 μg Mg	96 ± 11	93 ± 15
30 μg Pd + 2 μg Sr	102 ± 4	97 ± 11
30 μg Pd + 10 μg Ag	95 ± 9	98 ± 9
30 μg Pd + 2 μg Mg + 2 μg Sr	103 ± 10	103 ± 11
30 μg Pd + 5 μg Ag + 2 μg Sr	103 ± 3	105 ± 5

[a] Mean value ± SD (n = 4); [b] Results were obtained for urine samples spiked with 20 μg l^{-1} Se

/m , where V means the injection volume (10 μl) and m is the standard additions slope. The LOD, LOQ and m_0 values calculated for conditions with all the chemical modifiers studied are summarized in Table IV. Taking into account the sample dilution during the preparation step, the LOD and LOQ values obtained for Se in the original urine sample will be four times higher than those shown in Table IV. As the data demonstrate, the peak height measurements resulted in higher analytical sensitivity (lower characteristic mass) and higher detection power (lower limit of detection). The best values (lowest values of LOD and m_0) were achieved for the peak height measurements using Pd + Ag + Sr modifier. The characteristic mass value found out in our study (m_0 = 17 pg) was significantly lower than those published previously during determination of Se in urine by Tsalev *et al.* [12] in the presence of Rh chemical modifiers (m_0 = 49.3 pg), by Horng and Lin [9] for mineralized samples in the presence of Ni (m_0 = 39.8 pg), Laborda *et al.* [18] in the presence of Ni (m_0 = 35 pg), and Wang *et al.* [11] in the presence of Pd + Ni + NH_4NO_3 chemical modifier (m_0 = 44 pg). LOQ of 12 μg Se l^{-1} (in undiluted urine) is sufficient even for determination in urine of healthy population since typically urinary Se concentrations higher than 12 μg l^{-1} were reported in several geographical areas [1].

Table IV Comparison of analytical characteristics of Se determination in presence of all investigated chemical modifiers

Modifier	Peak height			Peak area		
	m_0 [a] pg	LOD [b] μg l^{-1}	LOQ [b] μg l^{-1}	m_0 [a] pg	LOD [b] μg l^{-1}	LOQ [b] μg l^{-1}
30 μg Pd	20 ± 2	1.6	5.4	38 ± 5	3.0	10
30 μg Pd + 2 μg Mg	20 ± 3	1.5	4.9	30 ± 5	2.0	6.7
30 μg Pd + 2 μg Sr	18 ± 3	1.7	5.7	37 ± 4	2.0	6.6
30 μg Pd + 10 μg Ag	22 ± 1	1.3	4.3	34 ± 1	1.9	6.5
30 μg Pd + 2 μg Mg + 2 μg Sr	18 ± 1	1.7	5.6	39 ± 3	2.0	6.5
30 μg Pd + 5 μg Ag + 2 μg Sr	17 ± 1	0.9	3.0	37 ± 2	1.5	4.9

[a] Mean ± SD (n = 4); [b] LOD (3σ), LOQ (10σ) values are reported for urine diluted in 1+3 ratio

Based on the above-presented results, Pd + Ag + Sr was finally selected as the most efficient chemical modifier for determination of urinary Se. In the presence of this modifier the effect of the matrix was under good control and the best sensitivity was achieved. Accuracy of the method was verified by analysis of two commercially available certified reference urine control samples Lyphochek Lot. No. 69011 and Seronorm™ Lot. No. 2525. In addition, accuracy was checked by comparison of the results obtained for determination of Se in two different urine samples of non-exposed general population by proposed method and by radiochemical neutron activation analysis (RNAA) [25]. In all the cases direct aqueous calibration using the equation $A = 2.40\ 10^{-3}\ (4.28\ 10^{-5})\ c$, (where A = absorbance evaluated from peak height measurements, c = concentration of analyte in $\mu g\ l^{-1}$; standard deviation of calibration slope is shown in parentheses) was used for quantification. As it follows from results in Table V, good agreement between the values were found in all the cases.

The precision of the method was examined by means of five replicate measurements of 15 $\mu g\ l^{-1}$ of Se in four times diluted urine. The relative standard deviation found out was 3.0 %.

Table V Determination of Se in certified reference urine samples and real urine samples (comparison of methods)

	Declared $\mu g\ l^{-1}$	Found[a] $\mu g\ l^{-1}$	Recovery %
Lyphochek 69011	61 ± 12	62 ± 6	102
Seronorm™ 2525	66.9 ± 7.1	63 ± 8	94
Urine sample 1	36.0 ± 1.6[b]	33 ± 3	92
Urine sample 2	20.0 ± 1.0[b]	18 ± 2	90

[a] Mean value ± 2 SD (n = 3).
[b] Results obtained by RNAA method

Conclusion

A critical comparative study of conventional (Pd, Pd+$Mg(NO_3)_2$), less commonly applied (Pd + Sr (nitrate), Pd + Mg + Sr (nitrate)) and new modifiers (Pd+Sr (iodide), Pd+Ag, Pd+Mg+Sr (iodide), Pd+Ag+Sr (iodide)) for the direct ETAAS determination of Se in human urine was carried out using D_2 BC. As it can be seen from the results presented, direct determination of Se in urine can easily be performed by ETAAS even with instrumentation equipped with D_2 BC under optimized operating conditions and using an appropriate chemical modifier. Pd+Ag+Sr was found to be the most convenient modifier for this purpose since the

effect of the matrix was under good control and the best LOD and acceptable recoveries were achieved in its presence. Using current instrumentation enabling faster heating of the atomizers, "excellent" isothermal conditions can be reached, so the effect of the matrix is not significantly different using both measurement modes. The use of peak height thus can be used with an advantage since it results in an increase in the detection power and sensitivity. The proposed simple method, where the sample preparation consists only in a mere dilution with water, can be thus applied to rapid and accurate determination of Se in urine at concentrations above 12 $\mu g\ l^{-1}$.

Acknowledgements

The study was developed thanks to financial support from the Ministry of Education, Youth and Sports of the Czech Republic (project No. 0021627502).

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