

## Speciation of inorganic selenium by solid phase extraction using nanozirconium oxide/boron oxide composite material

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**Abstract:** The speciation and preconcentration of selenium were carried out by solid phase extraction using nanozirconium oxide/boron oxide ( $ZrO_2/B_2O_3$ ). Electrothermal atomic absorption spectrometry (ETAAS) was used for detection. This preconcentration and/or speciation method depends on the retention of Se(IV) ions selectively as its ammonium pyrrolidine dithiocarbamate (APDC) complex on the sorbent. The effects of pH, flow rate, and volume of sample solution and eluent type were investigated for determining the optimum preconcentration and speciation conditions. The effects of other ions that may interfere with the determination and speciation of selenium were also investigated. Moreover, the analytical performance criteria of the method such as detection limit, quantification limit, linear working range, precision, and accuracy were also determined under the optimum experimental parameters. The analytical limit of detection for Se(IV) was  $1.21 \mu\text{g L}^{-1}$ . The accuracy of the method was checked by applying it to certified reference material (NIST 1643e) and spiked water samples. It was shown that the determination could be performed with a relative error of about 10%. The precision of the method was also at an acceptable level for analytical purposes for trace analysis (relative standard deviation <5%).

**Key words:** Speciation, preconcentration, selenium, solid phase extraction, nanometal oxide, electrothermal atomic absorption spectrometry

### 1. Introduction

In the last several decades, considerable interest has been shown towards the speciation of elements due to their different toxicity and mobility. The toxicity of different species depends on their chemical form.<sup>1</sup> Many trace elements such as selenium are essential for humans and thus are required for the basic physiological and metabolic processes, including enzyme processes.<sup>2</sup> Daily  $1 \mu\text{g}$  of selenium per kilogram of body weight for adults is accepted as the essential amount.<sup>3</sup> Selenium is necessary for the normal function of the immune system and thyroid gland. No clinical or biochemical signs of selenium toxicity were detected when daily mean selenium intake was  $4 \mu\text{g/kg}$  body weight.<sup>3</sup> Despite all the benefits of selenium, the maximum permissible level of it in drinking water is  $10 \mu\text{g L}^{-1}$ .<sup>3</sup> Selenium is both an essential and toxic trace element for humans. There is a specific concentration range that is beneficial for humans. Above this concentration range it is toxic. Selenium deficiency is an important problem as well as its toxicity. Therefore, to maintain human health and ecotoxicity, selenium levels have to be controlled in environmental samples such as drinking water. High selenium intake by means of nutrition increases the risk of stomach and bowel cancer.<sup>4</sup> Selenium arises in the environment in

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organic and inorganic forms. Selenium exists in water samples mainly as inorganic forms as Se(IV) and Se(VI). Inorganic selenium species are more toxic than organic selenium species. In addition, Se(VI) is more toxic than Se(IV).<sup>5</sup> Therefore, speciation analysis of selenium having different oxidation states is an important topic in various matrices and applications.<sup>6</sup> Speciation analysis is the quantitative determination of the individual chemical forms of analytes such as metals, metal compounds, organometals, and inorganic ions.<sup>2</sup> The total concentration of selenium, in general, cannot reflect the hazard or benefit of its different species. Therefore, it is important to develop simple, rapid, and efficient methods for monitoring the inorganic selenium species found in food and environmental samples.<sup>7</sup>

Electrothermal (ETAAS) and hydride generation (HGAAS) atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS) etc., which have low detection limits in the determination of Se, have been widely used.<sup>8–12</sup> However, in all methods, except HGAAS, mentioned above, only total metal concentrations can be determined directly (only Se(IV) by HGAAS). Thus, for speciation analysis, sample pretreatment steps such as separation or masking of species is usually needed before analyzing the samples by atomic spectroscopic method.<sup>2</sup>

In recent decades, many articles have been published on the development and application of speciation procedures by solid phase extraction (SPE). In this technique, one species is usually selectively retained on a solid phase.<sup>2,13</sup> The second possibility for speciation is to retain both of the species on the same sorbent but under different experimental conditions such as at different pH.<sup>14,15</sup> In some SPE speciation procedures, a reaction product of one species that will be retained on the solid material selectively was obtained by adding a reagent to the sample solution.<sup>16</sup> Alternatively, the solid phase material may be chemically or physically modified by a reagent to produce binding sites on the solid surface for the selective retention of one species.<sup>17–19</sup> In column SPE techniques, because a large volume of sample is passed through the column and the retained species is eluted by a small volume of eluent, preconcentration is also performed. Many different solid phases have been suggested and used in speciation studies to separate individual species selectively. Among these solid phases, nanomaterials (metal oxides), microorganism-loaded materials, chelating agent-loaded materials, and ion-imprinted materials have found wide application.<sup>2,20–25</sup>

In the present study, previously synthesized  $ZrO_2/B_2O_3$  nanocomposite material<sup>26</sup> was used for the first time as a sorbent for the speciation and/or preconcentration of Se(IV) from water samples. This material has been used before for As speciation<sup>27</sup> and also preconcentration of Co(II), Cu(II), and Cd(II)<sup>26</sup>. APDC was used as a complexing agent to improve selective retention of Se(IV) and thus to perform speciation.

## 2. Results and discussion

The efficiency of speciation and/or preconcentration methods based on SPE depends on various experimental parameters such as pH, flow rate of sample solution, sample volume, and other ions' effect. Therefore, these parameters should be optimized before real sample analysis. After optimization, the adsorption capacity of the sorbent was determined under the optimum conditions by applying a Langmuir adsorption isotherm. In addition, graphite furnace conditions, especially pyrolysis temperature, were also optimized before selenium determination.

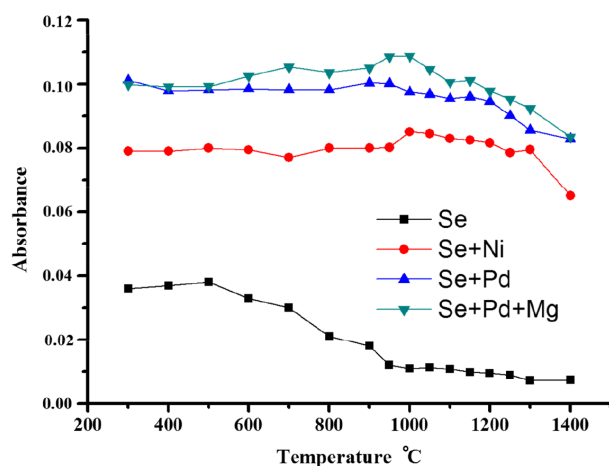
### 2.1. Optimization of furnace conditions

For volatile elements such as selenium, pyrolysis temperature is quite low (200–600 °C). Because APDC was used as a complexing agent, selenium could not be detected without a modifier. Therefore, the effect of modifiers

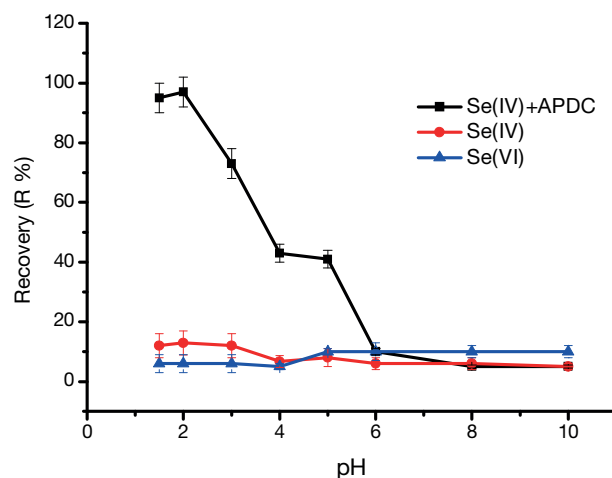
was also studied in this work. In order to determine a suitable pyrolysis temperature, the effects of 5  $\mu\text{g}$  of Pd, 5  $\mu\text{g}$  of Ni, and 5  $\mu\text{g}$  of Pd + 1  $\mu\text{g}$  of Mg mixture were investigated in the presence of 20  $\mu\text{g L}^{-1}$  Se(IV). As can be seen in Figure 1, while absorbance of selenium decreases at pyrolysis temperature above 500  $^{\circ}\text{C}$  without matrix modifier, it increases to about 1000  $^{\circ}\text{C}$  with all matrix modifiers studied. Because the integrated absorbance value with Pd + Mg mixture is higher than those of other modifiers, this mixture was selected as modifier for subsequent experiments. Due to the effect of atomization temperature on absorbance is low the literature value of 2600  $^{\circ}\text{C}$  was applied for the atomization temperature.<sup>28</sup>

## 2.2. Effect of pH

Because the solution and sorbent particles interface is affected by the pH of the solution, pH has an important role in all preconcentration and speciation methods based on SPE. Hydroxide and/or hydronium ions may change the charge of the surface of particles or nanoparticles. Therefore, different ions or molecules may retain at different pH values on nanoparticles. In order to demonstrate the effect of pH, the pH of model solutions (25 mL, containing 4  $\mu\text{g L}^{-1}$  Se) was adjusted to 1.5, 2, 3, 4, 5, 6, 8, and 10 by using dilute  $\text{HNO}_3$  and/or ammonia solution and the general preconcentration procedure (Section 3.4) was applied. It was found that while Se(IV) and Se(VI) alone were not retained on the column at all pH values studied, Se(IV)-APDC complex was retained sufficiently (recovery > 95%) at a pH range of 1.5–2 (Figure 2). Hence, pH 2 was selected as an optimum pH for the preconcentration/speciation of Se(IV) and Se(VI) by SPE for subsequent experiments.



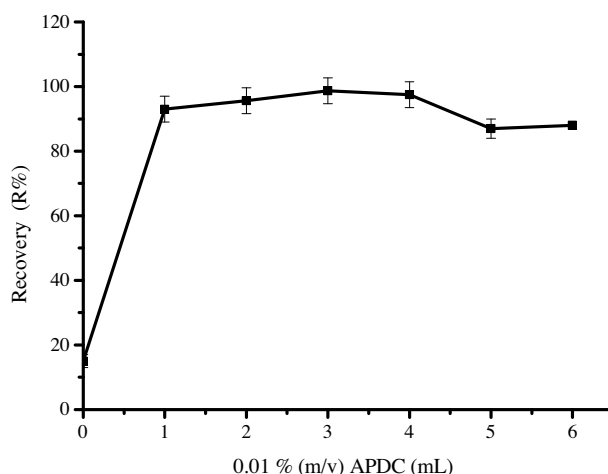
**Figure 1.** Pyrolysis curves for 20  $\mu\text{g L}^{-1}$  Se(IV) with and without matrix modifiers. Atomization temperature: 2600  $^{\circ}\text{C}$ ; Pd: 5  $\mu\text{g}$ ; Ni: 5  $\mu\text{g}$ ; 5  $\mu\text{g}$  Pd + 1  $\mu\text{g}$  Mg; injected volume: 20  $\mu\text{L}$ .



**Figure 2.** Effect of pH on the recovery of Se(IV) and Se(VI).

## 2.3. Effects of amounts of ammonium pyrrolidine dithiocarbamate (APDC)

The effect of amounts of APDC on the recovery of Se(IV) was also investigated (Figure 3). Se(IV) was recovered at about 18% in the absence of APDC. The recovery of Se(IV) reached about 95% with 1.0 mL of 0.01% (m/v) APDC. The recovery decreased after 4.0 mL. For all subsequent works 2.0 mL of 0.01% (m/v) APDC was used.



**Figure 3.** Effect of amount of APDC on the recovery of Se(IV).

#### 2.4. Effect of eluent type and concentration

In order to achieve high recovery, the eluent must be able to remove retained analytes from the sorbent easily and completely. Therefore, the type and concentration of eluent are also important parameters for preconcentration and speciation methods. Different analytes may have different releasing behavior with type and concentration of eluent. In order to determine a suitable eluent for removing Se(IV)-APDC complex from the nanosorbent, aqueous and ethanolic solutions of HCl and HNO<sub>3</sub> having various concentrations and various volumes were tested. For this purpose, 25 mL of model solutions containing 0.1 μg of Se(IV) and 2 mL of 0.01% (m/v) APDC were prepared. pH of the solutions was adjusted to 2.0 and the general column speciation and preconcentration procedure was applied. These results are given in Table 1. As can be seen from the results, 5 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub>, 10 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub>, and 10 mL of 2 M HNO<sub>3</sub> in ethanol solutions were optimal eluent solutions for desorption of Se(IV)-APDC complexes from the nanosorbent. Hence, 5 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub>, which results in a higher preconcentration factor, was selected as an optimum eluent for subsequent experiments.

**Table 1.** Effect of type and concentration of elution solutions on recovery of Se(IV).

Eluent	Recovery <sup>a</sup> (%)
10 mL of 2 mol L <sup>-1</sup> HCl	40 ± 4
10 mL of 2 mol L <sup>-1</sup> HNO <sub>3</sub>	47 ± 3
10 mL of 2 mol L <sup>-1</sup> HCl (in methanol)	62 ± 4
10 mL of 2 mol L <sup>-1</sup> HNO <sub>3</sub> (in methanol)	99 ± 5
10 mL of 1 mol L <sup>-1</sup> HNO <sub>3</sub> (in methanol)	96 ± 4
5 mL of 1 mol L <sup>-1</sup> HNO <sub>3</sub> (in methanol)	98 ± 4

<sup>a</sup>Mean ± standard deviation (N = 3).

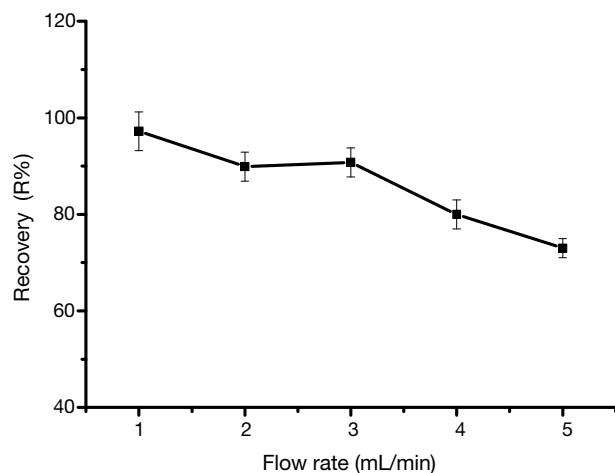
#### 2.5. Effect of flow rate on sample solution

The flow rate of sample solution affects both the retention of the analyte on the sorbent and the duration of the experiment. While increasing flow rate to shorten the duration, retention of the analyte decreases. However, when decreasing flow rate in order to increase retention, duration increases. Therefore, the effect of flow rate of sample solutions on the recovery of Se(IV)-APDC complex was examined in the range of 1–5 mL min<sup>-1</sup> under

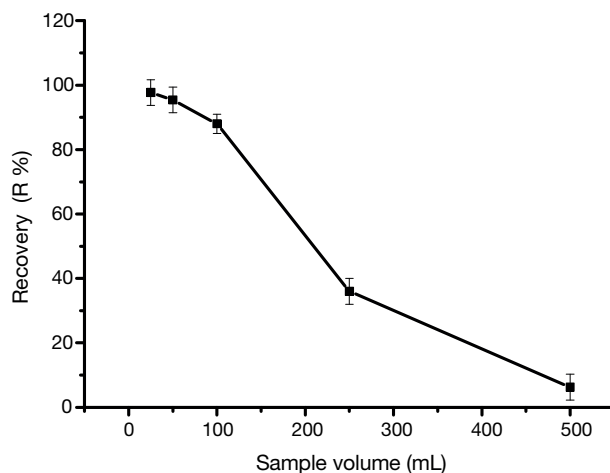
optimum conditions (pH 2; eluent, 5 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub>). Flow rate was adjusted by a peristaltic pump. As can be seen from Figure 4, Se(IV) was almost quantitatively recovered (above 95%) at 1 mL min<sup>-1</sup> sample solution flow rate. At the flow rates faster than this value, Se(IV) was not quantitatively recovered due to the lower contact time of Se(IV) with sorbent. For subsequent experiments, 1 mL min<sup>-1</sup> was chosen for the sample flow rate. Because eluent volume was relatively low (5 mL), the effect of eluent flow rate was not tested. In all experiments, 1 mL min<sup>-1</sup> eluent flow rate was used.

## 2.6. Effect of sample volume

In preconcentration studies, to achieve a high preconcentration factor, it is desired to use the maximum volume of sample and the minimum volume of eluent. In order to determine the optimum sample volume, sample volumes containing a constant amount of Se(IV) (0.1 μg) were tested in the range of 25–500 mL by applying a general preconcentration/speciation procedure (Section 3.4). Because the amount of Se(IV) is constant in all of the model solutions, its concentration is 4.0, 2.0, 1.0, 0.4, and 0.2 μg L<sup>-1</sup>, respectively for 25, 50, 100, 250, and 500 mL sample volume. The maximum and quantitative recovery (>95%) of Se(IV) can be obtained with only 50 mL of sample (Figure 5). Above 50 mL of sample volume (or below 2.0 μg L<sup>-1</sup> Se(IV)) the recovery of selenium decreased gradually. If 50 mL of sample solution was passed from the column and 5 mL of eluent solution was used for desorption of the Se(IV), the preconcentration factor of 10 was obtained for Se(IV). It can be concluded that by applying 50 mL of sample volume, 2.0 μg L<sup>-1</sup> Se(IV) could be determined.



**Figure 4.** Effect of the sample flow rate on the recovery of Se(IV).



**Figure 5.** Effect of the sample volume on the recovery of Se(IV).

## 2.7. Effect of foreign ions

In preconcentration, separation, and/or speciation studies with SPE, foreign ions can usually interfere with the determination in the SPE step and detection step. In the first step, other ions can also be retained on the sorbent, which may decrease the retention of the analytes. In the second step, other ions eluted together with the analyte may interfere with the atomic spectrometric determination. In SPE, because most of the other ions are separated from the analytes, interference in the detection step is generally low. However, other ions at high concentrations may prevent the retention of analyte onto the sorbent. For this reason, preconcentration and/or

speciation should be investigated in the presence of possible interfering ions of the water samples. In order to determine the effect of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Co}^{2+}$  as their nitrate or chloride salts on the recovery of Se(IV), they were added to 25 mL of model solutions containing 0.1  $\mu\text{g}$  of Se(IV) and 2 mL of 0.01% (m/v) APDC and a general preconcentration/speciation procedure (Section 3.4) was applied. The foreign ion concentration that caused  $\pm 10\%$  deviation in recovery of the Se(IV) is regarded as the tolerance limit. The effect of foreign ions on the recovery of Se(IV) and the tolerance limits are given in Table 2. The tolerance limit of  $\text{Fe}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Co}^{2+}$  is 5  $\text{mg L}^{-1}$ . This value is lower than those of the other ions studied. However, this concentration (5000  $\mu\text{g L}^{-1}$ ) is still 1250-fold the Se(IV) concentration (4  $\mu\text{g L}^{-1}$ ). Therefore, it can be concluded that the proposed nano- $\text{ZrO}_2/\text{B}_2\text{O}_3$  as solid phase extractor could be applied for the speciation and/or preconcentration of Se(IV) in water samples containing  $\text{mg L}^{-1}$  levels of other metal ions.

**Table 2.** Effect of foreign ions (Se(IV): 4  $\mu\text{g L}^{-1}$ ).

Foreign ions	Tolerance limits $\text{mg L}^{-1}$	Recovery, %
-	-	96 $\pm$ 4
$\text{Na}^+$	100	94 $\pm$ 4
$\text{K}^+$	100	95 $\pm$ 4
$\text{Ca}^{2+}$	100	93 $\pm$ 4
$\text{Mg}^{2+}$	100	93 $\pm$ 4
$\text{Ni}^{2+}$	5	95 $\pm$ 4
$\text{Zn}^{2+}$	25	93 $\pm$ 3
$\text{Cu}^{2+}$	5	102 $\pm$ 5
$\text{Cr}^{3+}$	25	98 $\pm$ 4
$\text{Al}^{3+}$	25	94 $\pm$ 4
$\text{Fe}^{3+}$	5	93 $\pm$ 4
$\text{Mn}^{2+}$	100	96 $\pm$ 3
$\text{Co}^{2+}$	5	98 $\pm$ 3

Mean  $\pm$  standard deviation (N = 3).

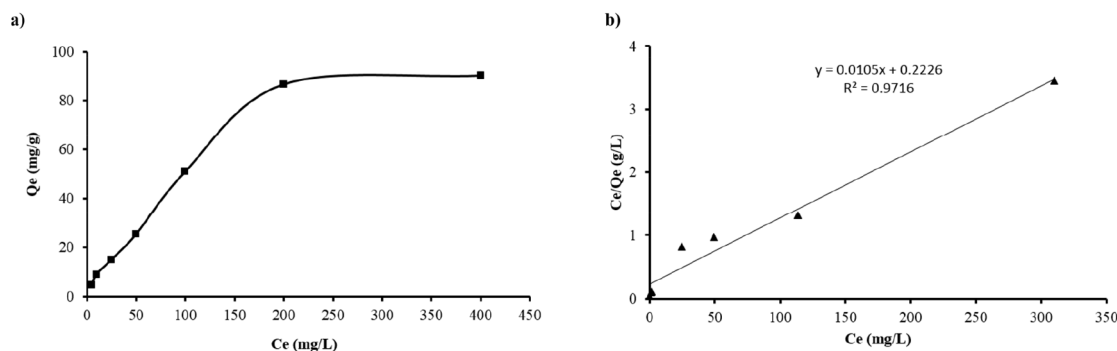
## 2.8. Adsorption capacity of nanosorbent

In order to determine the adsorption capacity of Se(IV) ions onto nano- $\text{ZrO}_2/\text{B}_2\text{O}_3$  composite material a batch technique was used. The Langmuir adsorption isotherm of Se(IV) was evaluated for adsorption mechanism and adsorption capacity. For this purpose the following experimental parameters were applied: amount of nanosorbent, 50 mg; working pH 2; volume of sample solution, 50 mL; Se(IV) concentrations, 5, 10, 25, 50, 100, 200, and 300  $\text{mg L}^{-1}$  with increasing amount complexation agent. The solutions were shaken for 2 h at 120 rpm at room temperature to reach equilibrium. Then the amount of free Se(IV) in each solution was determined by ETAAS. The data were plotted to obtain the Langmuir isotherm (Figure 6a) according to the Langmuir equation given below:

$$\frac{C_E}{Q_E} = \frac{C_E}{Q_0} + \frac{1}{Q_0 b},$$

where  $Q_0$  is the maximum amount of the Se(IV) adsorbed in milligram per gram of sorbent (capacity parameter) ( $\text{mg g}^{-1}$ ),  $C_E$  is the equilibrium concentration of Se(IV) in  $\text{mg L}^{-1}$ ,  $Q_E$  is the amount of Se(IV) adsorbed in

milligrams per gram of sorbent at equilibrium ( $\text{mg g}^{-1}$ ), and  $b$  is the Langmuir constant related to the affinity of binding sites.



**Figure 6.** (a) Langmuir adsorption isotherm plot of Se(IV); (b) Linearized Langmuir adsorption isotherm of Se(IV) on nano-ZrO<sub>2</sub>/B<sub>2</sub>O<sub>3</sub>.

From plot of  $C_e/Q_e$  versus  $C_e$  given in Figure 6b, the constant  $Q_0$  value was calculated from the slope. The value of  $Q_0$  was  $95.23 \text{ mg g}^{-1}$ . The Langmuir constant was  $0.047 \text{ L mg}^{-1}$  and  $r$  was 0.9716. It may be said that the adsorption of Se(IV) complex complies with the Langmuir isotherm ( $r = 0.9716$ ), indicating complete monolayer coverage on the surface.

## 2.9. Analytical performance of the proposed SPE method

Limit of detection (LOD), limit of quantification (LOQ), linear working range, precision, and accuracy are widely used for assessing the analytical performance of a method. To demonstrate the validity of a method, the values for these criteria should be determined. LOD and LOQ are calculated from the equations  $3s/m$  and  $10s/m$ , where  $s$  is the standard deviation of the blank signals and  $m$  is the slope of the calibration curve. For determining the instrumental LOD, the minimum amount of Se(IV) was added to 50 mL of water to obtain a blank solution. The blank solution was subjected to the general preconcentration procedure (Section 3.4) under the optimal conditions (pH 2, eluent: 50 mL of  $1 \text{ mol L}^{-1} \text{ HNO}_3$ ; sample flow rate:  $1 \text{ mL min}^{-1}$ ). In this procedure there is no preconcentration, because eluent volume is equal to sample volume (50 mL). From the 20 replicate measurements of this blank solution, standard deviation ( $s$ ) and the instrumental limit of detection (iLOD) were calculated (Table 3). By considering the preconcentration factor, the analytical limit of detection was calculated from the equation of  $i\text{LOD}/\text{PF}$ , where PF is the preconcentration factor (10 in this study).<sup>29,30</sup>

The precision of the method was determined by applying the procedure seven times under the optimum experimental conditions and calculated mean recovery and relative standard deviation. The accuracy of the method was checked by analyzing spiked water samples and certified reference material (NIST 1643e) containing  $11.97 \pm 0.2 \text{ } \mu\text{g L}^{-1}$  selenium. Because this concentration is relatively high for the ETAAS method, certified reference material (CRM) was used after dilution. For this purpose, a known amount CRM was taken and 2 mL of 5% (m/v) ascorbic acid was added to reduce any Se(VI) to Se(IV). Then 2 mL of 0.01% (m/v) APDC was added, pH was adjusted to 2, and the solution was completed to 25 mL with water, giving  $2.4 \text{ } \mu\text{g L}^{-1}$  selenium concentration. This sample was analyzed by the proposed method and selenium concentration was calculated by considering the dilution factor. All of the performance criteria of the method are given in Table 3. For checking the accuracy of the proposed method by using spiked samples, spiked solutions containing different amounts of

Se(IV) and/or Se(VI) species were prepared and subjected to the proposed procedure. The results are given in Table 4. As can be seen from Tables 3 and 4, relative error is less than 10% and the relative standard deviation is less than 5% for all samples. Both accuracy and precision values were at acceptable levels from the analytical point of view for trace analysis.

**Table 3.** Analytical performance criteria of the method.

Parameters		Value
Instrumental LOD, $\mu\text{g L}^{-1}$ (N = 20)		1.21
Analytical LOD (LOD/P.F.), $\mu\text{g L}^{-1}$ , (P.F. = 10)		0.120
Instrumental LOQ, $\mu\text{g L}^{-1}$ (N = 20)		3.60
Analytical LOQ (LOQ/P.F.), $\mu\text{g L}^{-1}$ , (P.F. = 10)		0.360
Precision	Mean recovery, %	96
	Relative standard deviation, %	$\pm 4$
	Number of experiments (N)	7
Calibration curve	Calibration curve equation ( $C_{Se(IV)} = \mu\text{g L}^{-1}$ )	$A = 0.0047C_{Se(IV)} + 0.0171$
	$r^2$	0.9996
	Linear working range, $\mu\text{g L}^{-1}$	0.36–40
Accuracy	NIST 1643 Certified value <sup>a</sup> $\mu\text{g L}^{-1}$	$11.97 \pm 0.2$
	Found value, $\mu\text{g L}^{-1}$ (N = 3)	$12.5 \pm 0.4$
	Relative error, %	+6

<sup>a</sup>) Composition of NIST 1643e: Al:  $141.8 \pm 8.6 \mu\text{g/L}$ , Cd:  $6.568 \pm 0.073 \mu\text{g/L}$ , Ca:  $32,300 \pm 1100 \mu\text{g/L}$ , Cr:  $20.40 \pm 0.24 \mu\text{g/L}$ , Co  $27.06 \pm 0.32 \mu\text{g/L}$ , Cu:  $22.76 \pm 0.31 \mu\text{g/L}$ , Fe:  $98.1 \pm 1.4 \mu\text{g/L}$ , Pb:  $19.63 \pm 0.21 \mu\text{g/L}$ , Se:  $11.97 \pm 0.2 \mu\text{g/L}$ , Mg:  $8037 \pm 98 \mu\text{g/L}$ , Mn:  $38.97 \pm 0.45 \mu\text{g/L}$ , Ni:  $62.41 \pm 0.69 \mu\text{g/L}$ , K:  $2034 \pm 29 \mu\text{g/L}$ , Na:  $20740 \pm 260 \mu\text{g/L}$ .

**Table 4.** Speciation with spiked solutions.

Sample	Added, $\mu\text{g/L}$		Found <sup>a</sup> , $\mu\text{g/L}$			Relative error, %		
	Se(IV)	Se(VI)	Se(IV)	Se(VI) <sup>b</sup>	Total Se	Se(IV)	Se(VI)	Total Se
1	4	-	$3.8 \pm 0.2$	-	$3.8 \pm 0.2$	-5.0	-	-5.0
2	4	4	$3.8 \pm 0.2$	$3.9 \pm 0.7$	$7.7 \pm 0.7$	-5.0	-2.5	-3.6
3	8	12	$7.8 \pm 0.4$	$12.9 \pm 0.9$	$20.7 \pm 0.8$	-2.5	+7.5	+3.5
4	-	4	-	$4.2 \pm 0.2$	$4.2 \pm 0.2$	-	+4.6	+4.6

<sup>a</sup> Results are mean of three replicates  $\pm$  standard deviation. <sup>b</sup> Calculated value ( $\text{Se(VI)} = \text{Total Se} - \text{Se(IV)}$ ).

The linear working range of the method is 0.36–40  $\mu\text{g L}^{-1}$ . As a lower limit of the linear working range, LOQ value is taken.

## 2.10. Application of the proposed method

The proposed preconcentration and/or speciation method for selenium was applied to model solutions prepared synthetically, tap water, and hot spring waters. Spiked water samples with selenium species (Se(IV) and Se(VI)) were also analyzed to check the accuracy once again. The results are shown in Table 5. As can be seen, there is good agreement between spiked and found values (relative errors < 10%). These results indicate the applicability of the proposed speciation method to water samples, including thermal waters.



**Table 5.** Determination of selenium species in various samples.

Sample	Added, $\mu\text{g L}^{-1}$		Found <sup>a</sup> , $\mu\text{g L}^{-1}$			Relative error, %		
	Se(IV)	Se(VI)	Se(IV)	Se(VI) <sup>b</sup>	Total Se	Se(IV)	Se(VI)	Total Se
Beypazarı thermal water (Tahtalı-Dutluk)	-	-	$3.15 \pm 0.04$	$1.56 \pm 0.3$	$4.7 \pm 0.3$	-	-	-
	4	4	$6.8 \pm 0.7$	$5.7 \pm 0.7$	$12.5 \pm 0.4$	-4.9	+2.5	-1.6
Çamlıdere dam water	-	-	$3.8 \pm 0.2$	$0.6 \pm 0.4$	$4.4 \pm 0.4$	-	-	-
	4	4	$8.4 \pm 0.3$	$4.2 \pm 0.9$	$12.6 \pm 0.9$	+7.7	-8.7	+2.1
Tap water	-	-	$0.50 \pm 0.06$	$0.05 \pm 0.09$	$0.55 \pm 0.07$	-	-	-
	4	4	$4.1 \pm 0.1$	$3.8 \pm 0.2$	$7.9 \pm 0.1$	-8.9	-6.2	-7.5

<sup>a</sup> Mean  $\pm$  standard deviation (N = 3). <sup>b</sup> Calculated value (Se(VI) = Total Se – Se(IV)).

### 3. Experimental

#### 3.1. Apparatus

A Varian electrothermal atomic absorption spectrometer (Palo Alto, CA, USA) AA240 model equipped with pyrolytic graphite tube (Varian P/N-63-100012-00) with platform and Zeeman background corrector was used. A PSD-120 model autosampler was used for the sample changer and injection system. As a line source, a selenium hollow cathode lamp (Varian) with a lamp current of 15 mA was used. The optimum operating conditions used for ETAAS are given in Table 6. A WTW 720 model pH meter (Weilheim, Germany) was used for all pH measurements of samples. A peristaltic pump (Watson Marlow 323, Wilmington, MA, USA) was used for adjusting the flow rate of sample solution.

**Table 6.** Experimental conditions for selenium determination by ETAAS.

Parameters	Value
Wavelength, nm	196.0
Slit width, nm	0.5
Lamp current, mA	15
Background correction	Zeeman
Inert gas	Argon (flow rate: 0.3 L/min)
Matrix modifier Injection volume	5 $\mu\text{g}$ of Pd + 1 $\mu\text{g}$ of Mg 20 $\mu\text{L}$
Drying temperature, °C	95 (ramp time: 40 s; hold time: 10 s) 120 (ramp time: 10 s; hold time: 15 s)
Pyrolysis temperature, °C	1000 (with matrix modifier) (ramp time: 5 s; hold time: 3 s)
Atomization temperature, °C	2600 (ramp time: 3 s; hold time: 2 s)
Cleaning temperature, °C	2700 (ramp time: 1 s; hold time: 3 s)

#### 3.2. Reagents and solutions

Analytical reagent grade chemicals were used unless otherwise stated. Ultrapure water (18.3 M $\Omega$  cm) was used for the preparation of all solutions. Se(IV) and Se(VI) standard solutions and model solutions were prepared by dilution of stock solutions of Se(VI) and Se(IV) (1000  $\mu\text{g mL}^{-1}$ , Merck, Darmstadt, Germany). L(+) ascorbic acid (Carlo Erba, Milan, Italy) was used as a reducing agent. Hydrochloric acid (37%, Merck), nitric acid (65%, Merck), ammonia solution (25%, Merck), and methanol (Merck) were used. For washing the laboratory glassware 5% nitric acid solution was used before every use.

Nano-ZrO<sub>2</sub>/B<sub>2</sub>O<sub>3</sub> was synthesized in our laboratory for the first time and characterized by various characterization techniques such as scanning electron microscope (SEM), transmission electron microscope (TEM), and X-ray diffraction (XRD) method.<sup>26</sup> For this purpose, 10 g of H<sub>3</sub>BO<sub>3</sub>, 5 g of Zr(OCl)<sub>4</sub>, 50 mL of

ethanol, and 1.5 mL of Triton X-114 were stirred and sonicated in an ultrasonic bath. The mixture was dried in an oven at 70 °C for about 1 h and then heated at 850 °C for about 2 h. The obtained material was ground to obtain powder product. As a result of a previous characterization study<sup>26</sup> it was found that particles of material consist of mainly crystalline B<sub>2</sub>O<sub>3</sub> and ZrO<sub>2</sub> and are very fine (the grain size is below 100 nm).<sup>26</sup>

### 3.3. Column preparation

A glass column having a reservoir of 250 mL on top of the column (15 cm length and 8 mm i.d.) was used as the solid phase extraction system, and 200 mg of nano-ZrO<sub>2</sub>/B<sub>2</sub>O<sub>3</sub> composite material was placed onto glass wool already placed in the column. In order to prevent disturbance during the sample passage through the sorbent, another small piece of glass wool was placed on top of the sorbent. The sorbent was conditioned prior to sample passage by treating with some water adjusted to the experimentally found optimum pH value. After each use, the sorbent was washed with eluent and water, consecutively. The sorbent in the column was stored in water until the next experiment. It was observed that the column could be reused up to 100 times.<sup>26</sup>

### 3.4. Speciation and preconcentration procedure

Preliminary experiments showed that selenium species (Se(IV) and Se(VI)) did not adsorb directly to nano-ZrO<sub>2</sub>/B<sub>2</sub>O<sub>3</sub>. Therefore, a suitable complexing agent was investigated to provide sufficient retention of selenium species. From the literature survey it was found that APDC may be a suitable complexing agent for Se(IV).<sup>11</sup> By using APDC, Se(IV) was retained quantitatively as its Se(IV)-APDC complex at about pH 2, whereas Se(VI) was not retained sufficiently. The following speciation procedure was applied: (1) Sample was passed through the column in the presence of APDC at optimum conditions (pH 2; flow rate, 1 mL min<sup>-1</sup>; complexing agent, 2 mL of 0.01% (m/v) APDC) determined experimentally in order to retain Se(IV)-APDC complex. Then Se(IV)-APDC complex was eluted from the column with a suitable eluent (5 mL of 1 mol/L HNO<sub>3</sub>) and only Se(IV) was determined by ETAAS in this eluent. (2) Sample was treated for 30 min with a reducing agent (L(+) ascorbic acid) to reduce all Se(VI) to Se(IV). Then procedure (1) was applied to determine total selenium. (3) Se(VI) content was calculated by subtracting the result of (1) from the result of (2).

For the above procedure, 25 mL of model selenium sample (containing 4 µg L<sup>-1</sup> Se(IV) and/or Se(VI)) was treated with 2 mL of 5% (m/v) L(+) ascorbic acid for 30 min. Then 2 mL of 0.01% (m/v) APDC was added to the sample. The pH value of Se(IV)-APDC solution was adjusted to 2 by adding dilute HNO<sub>3</sub> and/or ammonia solution and passed through the column at a flow rate of 1 mL min<sup>-1</sup>. The retained Se(IV)-APDC complexes were eluted by 5 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub> (in methanol) at a flow rate of 1 mL min<sup>-1</sup>. In this procedure analyte was also preconcentrated and separated from the sample matrix by extracting the analyte from 25 mL of sample into 5 mL of known eluent (preconcentration factor is 5 for this model solution study).

## 4. Conclusions

The proposed preconcentration and/or speciation method for Se(IV) and Se(VI) is simple, rapid (about 90 min for 25 mL of sample), precise (RSD: 4%), and accurate (relative errors < 10%). The speciation of different oxidation states of selenium can be performed by only two SPE methods: (1) by subjecting the sample to the procedure directly for Se(IV) determination and (2) by subjecting the sample to the procedure after reduction of Se(VI) to Se(IV) for total selenium determination. Se(VI) can be calculated easily from the difference.

Although the preconcentration factor is relatively low (only 10-fold), the recovery is sufficient for analytical purposes and speciation of Se(IV) and Se(VI), which is the main purpose of this study, could be performed easily and successfully. In this method, possible precipitation of metal hydroxides is prevented due to allowing an acidic pH value (pH 2) for SPE. The analytical performance of the proposed method (LOD, precision, accuracy etc.) is comparable with that of the other speciation method in the literature (Table 7). The adsorption capacity of the nanosorbent is also much higher than those of the articles given in Table 7.

**Table 7.** Comparison of the proposed method with some of the methods reported in the literature.

Method	Adsorbent	Species	Sample	pH	LOD	Adsorption	References
					$\mu\text{g/L}$	capacity mg/g	
ETAAS	Dowex 1X2	Se(IV)	Water	$\geq 5$	0.006	0.37	8
ICP-OES	CTAB <sup>a</sup> modified alkyl silica	Se(VI)	Water	2.0–10.0	0.10	5	10
ETAAS	TiO <sub>2</sub>	Se(IV)	Water and food	1.0–2.5	0.01	-	11
ETAAS	Diaion HP-2MG	Se(IV)	Water	2.0–6.0	0.06	5.2	31
FI-HGAAS	EVA <sup>b</sup>	Se(IV)	Water	8.0–10.0	0.03	-	32
ETAAS	ZrO <sub>2</sub> /B <sub>2</sub> O <sub>3</sub>	Se(IV)	Water	1.5–2	0.12	95.23	This study

<sup>a</sup> Cetyltrimethylammonium bromide. <sup>b</sup> Ethylvinylacetate.

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