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Preconcentration and Speciation of Se with Flow Injection Analysis Hyphenated in line to ETAAS

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Abstract

In the present work a reliable and highly sensitivity method for selenium speciation, based on sorbent pre-concentration using a mini column of anionic exchanger (LC-SAX) coupled in line to Graphite Furnace Atomic Absorption Spectrometry (GFAAS) is described. Chemometric approaches for those factors that exert influence on pre-concentration has been employed using a full 2^3 factorial design. Under acid medium (pH 2) only Se(VI) was determined while in the pH range of 6 up to 8, both Se(IV) and Se(VI) can be determined. Unlike from some previously published methods for selenium speciation, in which Se(VI) is determined by subtracting Se (IV) from total selenium, in the present work a system with three equations was proposed and this system was based on the principle of additivity of absorbances. The limit of detection and quantification for Se(IV) were 3.1×10^{-2} μg L^{-1} and 1.0×10^{-1} μg L^{-1} , respectively and for Se(VI) were 4.9×10^{-2} μg L^{-1} and 1.6×10^{-1} μg L^{-1}, respectively

Keywords Flow injection, Selenium, Atomic Absorption Spectrometry.

1. Introduction

Selenium is part of the active centers of selenoenzimes and, therefore, is an essential element for humans [1]. Some researches relate this element with the prevention of some types of cancer [2]. It is known to be essential for biological systems, whilst it is toxic at levels of only three to five times above bio-essential concentrations. In general, inorganic forms of selenium are more toxic than organic forms [3]. Selenium can be found in natural water, being the maximum allowed level, according to WHO, 10 μg L^{-1}, and in pharmaceuticals selenium complex with aminoacids (selenium chelates).

Several techniques are usually employed for selenium determination including inductively-coupled plasma mass spectrometry (ICP-MS) [4], inductively-coupled plasma atomic emission spectrometry (ICP-AES)5, UV-Vis spectrometry [6], X-ray fluorescence [7], voltametry [8] and hydride-generated flame atomic absorption spectrometry (HG-FAAS) [9,10].

The electrothermal atomic absorption spectrometry (ET-AAS) presents good performance [11] for metals determination in the range of μg L^{-1} nevertheless; sometimes the preconcentration is necessary due the low levels of selenium in the samples, generally about ng L^{-1}.

According to literature [3], the solid phase extraction (SPE) is one of the most techniques used for selenium preconcentration. The main advantages are the high preconcentration factor, ease to automation and coupling to several detection techniques.

The SPE can be performed on-line through columns [12] coupled directly by flow system to detectors. For instance, selenium determination using SPE coupled to FAAS has been related since 1983 [13]. The SPE-FAAS coupling is very simple because both techniques are of continuous flows.

The preconcentration using flow analysis coupled to ET-AAS is more complex because this hyphenation occurs in a discontinuous, parallel and synchronized way with the atomization step. However, it can be getting the best limits of detection.

The first preconcentration coupled on-line to ET-AAS was published in 1990 [14]. A similar publication was carried out by Queiroz et al (2002) [15] for copper determination, where they used the term in-line instead of on-line to refer to this type of coupling.

Some advantages of in-line coupling may be mentioned: low reagent consumption; increasing the analytical frequency; low risk of sample contamination and loss of analyte. Besides the advantages, there are few publications using this system for selenium speciation. The interface on this type of coupling takes place between the atomic absorption spectrometer autosampler arm and the polyethylene tube of FIA system. Some authors have used the FIA system to inject the sample on graphite tube, being the sample volume controlled by a sample loop [16, 17]. In a recent publication, the sample volume was controlled by the syringe pump of the autosampler of the atomic absorption spectrometer [18].

The aim of this work was to develop a preconcentration system in-line to ET-AAS configuration. The study of the factors that exert influence on solid phase extraction was performed by using a 2^3 full factorial design, while Doehlert matrix associated with response surface methodology was employed for final optimization. The method was applied on water and pharmaceutical samples and present satisfactory results.

The main advantage of the present method is based on the use of the principle of additivity of absorbance for the calculation of selenium concentration, unlike of some previous studies, where selenium (IV) is calculated by difference between total selenium and selenium (VI). Thus, using the proposed method, none reduction step is necessary.

2. Experimental

2.1. Instruments and apparatus

An electrothermal atomic absorption spectrometer Zeiss AAS 5 EA (Germany) was used, furnished with a transverse-heated of

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the graphite tube in combination with the deuterium continuum background corrector, and an MPE 5 autosampler. A selenium hollow cathode lamp (AnalytiKjenaAG) operating at 6 mA and 196 nm was used as radiation source; graphite tube with L´vov platform also was used. The heating program was used as follow: 110 ^{o}C during 30 s for dry; 1100 ^{o}C and 30 s for pyrolysis, 2100 ^{o}C and 5 s for atomization and 2600 ^{o}C for 3 s for cleaning, these values were adapted from Saygi et al. (2007) [19].

The FIA system for selenium preconcentration was coupled to the atomic absorption spectrometer autosampler. An 8-channels peristaltic pump was used and 2.06 mm i.d. of Tygon® tubes for fluid flowing; three solenoid valves of three ways and 0.8 mm i.d. polyethylene tubes to interconnect the system, and a microcomputer for data acquisition and system controlling [20].

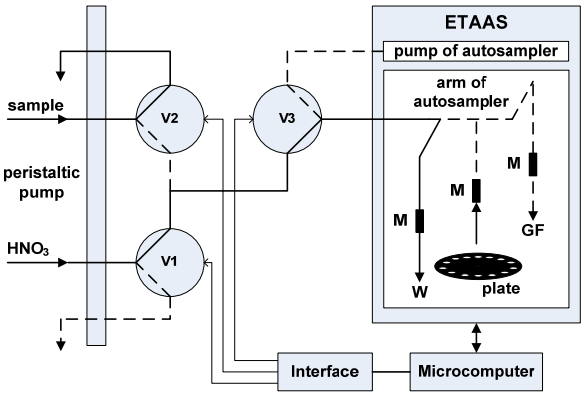

Figure 1. Squematic diagram of in-line preconcentration system to ET-AAS. Components: interface-microcomputer to solenoid valves controlling and data acquisition. HNO_3 0.1 mol L^{-1} was used for cleaning of the minicolumn, the FIA system connections were done using Tygon® and polyethylene tubes. M is an anion exchange minicolumn (15 mm, 2.06 mm i.d.); V1..3, solenoid valves of three ways; W, waste and GF, graphite furnace.

2.2. Reagents

All solutions used on this work were prepared with analytical grade chemical reagents as well as with water obtained from a Milli-Q purification system (Millipore). The glassware was cleaned by keeping at a 10% (v/v) HNO_3 solution, and rinsed with deionized water prior use. The selenate and selenite stock solutions were prepared by appropriate dilutions of Na_2SeO_4 (Alfa Aesar®) and SeO_2 (Titrisol® Merck), respectively. Buffer solutions were prepared from boric acid (Merck) by dissolving appropriate masses in pure water followed by alkaline pH adjustment, with sodium hydroxide. Nitric acid (Merck) to prepare the eluent solution and for pH adjustment was used. The solutions of concomitants ions Cl^-, NO_2^-, NO_3^- were prepared by dilution of NaCl, $NaNO_2$ and $NaNO_3$ respectively; the Al^{3+} and Mn^{2+} were prepared by dilutions of Titrisol® (Merck) stock solution. The concentration of concomitants were based on maximum limit proposed by Brazilian legislation [21], and in the concentration determined in water of Furnas reservoir located close to the Alfenas city (Brazil). Thus, the following concentrations were studied: Cl^- - 1.0, 125 and 250 mg L^{-1}; NO_2^- - 0.035, 0.500 and 1.00 mg L^{-1}; NO_3^- - 5.0; 10.0 and 250 mg L^{-1}; Al - 0.05, 0.10 and 1.40 mg L^{-1} ; Mn - 0.05 , 0.1 and 0.14 mg L^{-1}.

The chemical modifier $Pd(NO_3)_2$ 1005 mg L^{-1} (Aldrich Chemical), was utilized without later dilution, and the anion exchange resin LC-SAX (SUPELCO®) was used as sorbent.

2.3. Minicolumn preparation and flow manifold.

A filled minicolumn (Tygon® tube of 2.06 mm i.d. and 15 mm of length) with anion exchange resin LC-SAX (SUPLECO®) was built for in-line preconcentration system coupled to ET-AAS. This resin is a quaternary ammonium bonded to silica and Cl^- is counter ion. To avoid loss of sorbent glass wool was utilized and the connection to FIA system was carried by coupling a polyethylene tube (i.d. 0.8 mm). They were inserted on both ends of the solid support.

The coupling between the FIA system and the atomic absorption spectrometer (Figure 1) was accomplished in two ways: by the autosampler arm containing the minicolumn, and by the solenoid valve number 1 with the syringe pump.

The preconcentration FIA system was operated in three steps and synchronized with the heating program. The minicolumn cleaning happened in the first step; the preconcentration in the second, and the selenium elution in the third step.

Initially the minicolumn was connected to the peristaltic pump; the autosampler arm was in discard position; the valve 1 and 3 were switched on, flowing a cleaning solution (HNO_3 0.1 mol L^{-1}) at a flow rate of 4 mL min^{-1} for 60 s, this solution also conditions the column for the next sample. Further, the valves 1 and 2 were switched on to preconcentrate the analyte for 90 s.

On step 3, all the valves were off, and the FIA system was connected to the syringe pump of autosampler. The autosampler was started and 20 µL of eluent were carried out by minicolumn, 5 µL of palladium nitrate were also collected but this solution didn't have any contact with the exchange resin. The autosampler arm was positioned to the graphite furnace where the eluate was discharged. The eluent passed twice by the column (aspirated and dispensed), what increased the power of elution.

Finally, the autosampler arm was positioned to the initial stage, and all procedure was performed for each sample automatically.

2.4. Optimization study of the selenium preconcentration

The proposed method was optimized using multivariate statistical techniques. The main advantage of this statistical tool with respect to univariate optimization study is the analysis of the effects with their interactions. Thus, firstly a factorial design 2^3 was proposed for testing the effects of each factor as well as the effects of interactions. Then, a final optimization was performed using the response surface methodology. The results were analyzed with the help of STATISTICA 6.0 software.

2.5 Accuracy study and application

The proposed method was applied in reference certified material (RCM) (Dolt-4 fish liver, NRCC) liver, in which 100 mg of RCM were weighted, added 6 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%). The sample was maintained overnight. Next, it was decomposed in a microwave oven Ethos Plus (Millestone®) with a heating ramp starting from room temperature up to 200 ^{o}C for 10 minutes and after hold in 200 ^{o}C for more 10 minutes. Afterwards, the sample decomposed was heated in hot plate almost to dryness, and the volume was made up to 250 mL with deionized water. From this solution, two aliquots were taken, and pH values adjusted to 2.0 and 8.8. Finally, the preconcentration and determination procedure was performed under optimized conditions. The same microwave digestion procedure was used for pharmaceuticals samples. Water samples were determinate without previous preparations.

3. Results and discussion

3.1 Optimization

The 2^3 full factorial design, used to determine which factors are significant in the system, definitions of the factors and their levels are summarized in Table 1. The experiments in duplicate were carried out using 6.0 mL of selenite standard solution in a concentration of 5 µg L^{-1}.

Table 1 - Factors, levels and results obtained for the 2^3 factorial designs.

Factors				**Levels**	
				(-) Low	(+) High
pH				8.0	9.5
Preconcentration flow rate (PFR/mL min^{-1})				2.0	4.0
Eluent concentration (EC)/mol L^{-1}				1.0	2.5
Runs	1	2	3	**Absorbance (area)**	
1	–	–	–	0.366	0.416
2	+	–	–	0.334	0.354
3	–	+	–	0.529	0.575
4	+	+	–	0.475	0.460
5	–	–	+	0.410	0.430
6	+	–	+	0.692	0.737
7	–	+	+	0.569	0.632
8	+	+	+	0.589	0.647

Table 2 - Doehlert design and results obtained for the flow preconcentration system coupled to ETAAS for selenium determination. The first values represent the real values of the factors while the values between parentheses are the coded values from the Doehlert design for three factors. Preconcentration flow rate (PFR) and eluent concentration (EC).

Factor	**Level**						
EC	0.8	1.3	1.8	2.3	2.8	3.3	3.8
PFR		1.0	2.5	4.0	5.5	7.0	
pH			7.0	9.5	12.0		

Runs	**PFR (mL min^{-1})**	**EC (mol L^{-1})**	**pH**	**Absorbance (area)**
1	4.0(0)	2.3(0)	9.5(0)	0.4658
1	4.0(0)	2.3(0)	9.5(0)	0.4235
1	4.0(0)	2.3(0)	9.5(0)	0.4282
2	7.0(1)	2.3(0)	9.5(0)	0.1816
3	5.5(0.5)	3.8(0.866)	9.5(0)	0.1089
4	5.5(0.5)	2.8(0.289)	12.0(0.817)	0.0964
5	1.0(-1)	2.3(0)	9.5(0)	0.4634
6	2.5(-0.5)	0.8(-0.866)	9.5(0)	0.1677
7	2.5(-0.5)	1.8(-0,289)	7.0(-0.817)	0.2570
8	5.5(0.5)	0.8(-0.866)	9.5(0)	0.1333
9	5.5(0.5)	1.8(-0.289)	7.0(-0.817)	0.0669
10	2.5(-0.5)	3.8(0.866)	9.5(0)	0.4989
11	4.0(0)	3.3(0.577)	7.0(-0.817)	0.3773
12	2.5(-0.5)	2.8(0.289)	12.0(0.817)	0.1001
13	4.0(0)	1.3(-0.577)	12.0(0.817)	0.0998

The Pareto Chart (Figure 2), obtained from analysis of variance (ANOVA) of the factorial design, represents the significance factors and their interactions using confidence interval at the 95% level, defined by the vertical line. Horizontal bars higher than the vertical line establish the significance of factors. Therefore, as verified, all the factors and their interactions were statistically significant, being the eluent concentration (EC) one that exerts greater influence on the system, while sample pH has less effect on it. All the factors have positive estimated effect, indicating that, higher analytical responses can be achieved when there is an increase in the level of these factors. Hence, a final optimization would be possible by increasing the levels of these three factors. Thus, a Doehlert design was built [22], where the eluent concentration, in which exerts greater influence on the system was chosen as the factor with the largest number of levels in the Doehlert design (Table 2).

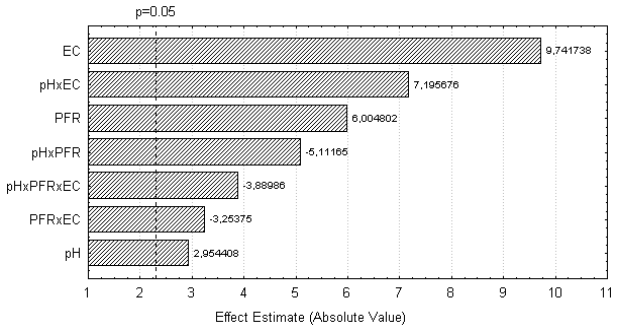

Figure 2. Pareto Chart showed the effect estimated for the factors and for their interactions. EC = eluent concentration; PFR = preconcentration flow rate.

On the other hand, only three levels were employed for sample pH. The center point was performed in triplicate. From Doehlert design, the quadratic model for analytical response was evaluated, represented by following equation:

$$\begin{matrix} Absorbance \\ (\text{peak area}) \end{matrix} = \begin{cases} -3.35511 - 0.04783\ PFR - 0.01296\ PFR^2 \\ +0.94312\ EC - 0.08124\ EC^2 + 0.62472\ pH - 0.03406\ pH^2 \\ -0.03951\ PFR\ EC + 0.02033\ PFR\ pH - 0.03739\ EC\ pH \end{cases} \quad (1)$$

ANOVA data showed that quadratic model (eq. 1) did not present lack of fit [($MS_{lack\ of\ fit}/MS_{pure\ error}$) = 7.21 < $F_{3,2}$ = 19.16 (table critical value)]. When the $MS_{lack\ of\ fit}/MS_{pure\ error}$ ratio is higher than the table critical *F* value, the model presents lack of fit and is not suitable [23].

In order to determine the nature of the stationary points of function, the Lagrange's criteria, a mathematical procedure was performed. This is based on the calculation of Hessian's determinants.

The Hessian determinants of a function (*PFR*, EC and pH) were calculated by using expressions [22] to determine the values of Δ_1; Δ_2 and Δ_3. There is a maximum point in quadratic model, when $\Delta_1 < 0$; $\Delta_2 > 0$ and $\Delta_3 < 0$, being the values found: –0.02592, 0.00265044 and –0.0003024 respectively, confirming the presence of maximum points.

In order to determine these maximum points, a tentative for solving a matrix system from quadratic model was carried out. However, as the matrix determinant was near zero it was not solved. For this reason, the PFR factor was fixed at 4.0 mL min^{-1}. This value was chosen to provide satisfactory sample throughput and absence of leakages in the mini-column. Then, a new equation with two factors was obtained (eq. 2), in which provided two optimum points of system: 2.8 mol L^{-1} for EC and pH 8.8 for sample. These values were established for the method.

$$\begin{matrix} Absorbance \\ (\text{peak area}) \end{matrix} = \begin{cases} -3.75379 + 0.78508\ EC - 0.08124\ EC^2 \\ +0.70604\ pH - 0.03406\ pH^2 - 0.03739\ EC\ pH \end{cases} \quad (2)$$

A graphic presentation of equation 2, can be showed by the pH and EC response surface (Figure 3).

On the choice of buffering system, the absorbance signals of buffered samples were compared to the samples signals without buffering, i.e., the pH adjustment was made with the addition of NaOH. The obtained absorbance signal with buffered solution in 0.005 mol L^{-1} borate buffer was only 5% lower than the analytical signal without buffer. However, when the ammonia buffer (in the same concentration) was used, the signal loss was 38.2 %. This way, the borate buffer was chosen in the present study.

In order to match the perfect synchronism between the heating

program and preconcentration system, a sample volume of 6.0 mL was used. Nitric acid was chosen as eluent in according to previous published works [24].

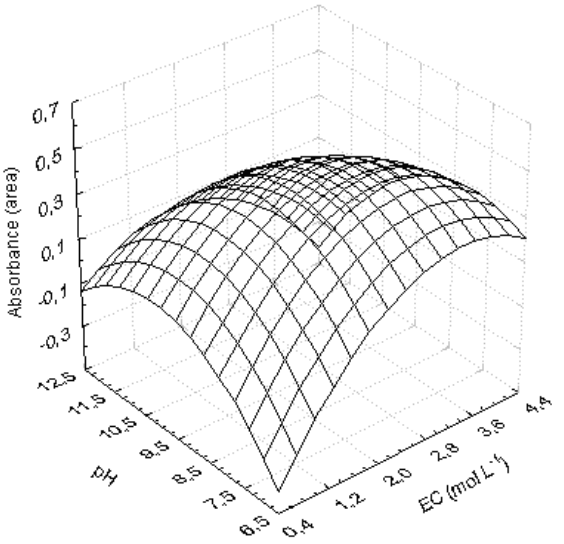

Figure 3. Surface response obtained from Doehlert design employed for optimization of sample pH and eluent concentration (mol L^{-1}).

3.2 Effect of sample pH in the selenium speciation

The pH of selenite and selenate solutions at 10 µg L^{-1} concentration was ranged from 1 up to 8 (Figure 4). This study was based on ionization constants of both species, K_1=2.7x10^{-3} and K_2=2.5x10^{-7} for selenous acid, and K_2=2.0x10^{-2} for ionization constant of selenic acid [25]. This study showed that only selenate anions are preconcentrated in the exchange resin for pH upon 2.0. However, selenite is preconcentrated in a significant way above pH 6.0. Therefore, two values of pH were chosen for speciation of these two species: pH 2.0 for selenate and pH 8.8 for selenite.

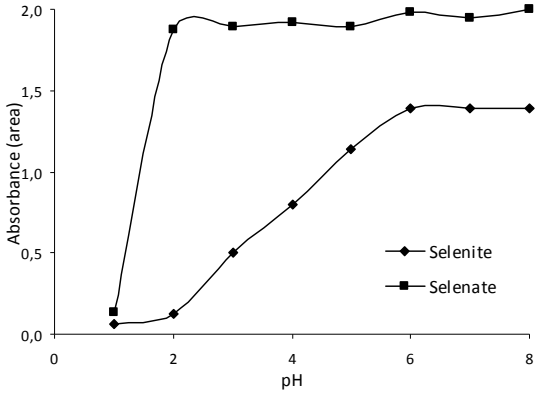

Figure 4. pH study of selenite and selenate anions in the pre-concentration system.

3.3 Analytical curves of speciation system

Based on the additivity principle of absorbance and in according to pH studies, it can be pointed out that a sample in an alkaline medium containing selenite and selenate, originates an absorbance signal that is a sum of each species. Therefore, with the aim of obtaining a correlation between the selenium species absorbance signals, three analytical curves were built. One of them for selenate, in pH 2.0, and the other two were built for selenite and selenate in pH 8.8.

Therefore, the selenite and selenate concentration were obtained by solving a system of three linear equations and two unknowns from the analytical curves. This way, in an acid solution the signal is governed by equation 2.1 and in an alkaline solution it is by equation 2.2. The selenate (eq. 2.3) and selenite (eq. 2.4) concentrations were obtained by these equations.

$$A_1 = m_1 C_{Se(VI)} + a_1 \quad (2.1) \qquad A_2 = \left(m_2 C_{Se(VI)} + a_2\right) + \left(m_3 C_{Se(IV)} + a_3\right) \quad (2.2)$$

$$C_{Se(VI)} = \frac{A_1 - a_1}{m_1} \quad (2.3) \qquad C_{Se(IV)} = \frac{m_1}{m_2 m_3}\left(\frac{A_2 - a_2 - a_3}{A_1 - a_1}\right) \quad (2.4)$$

where: $C_{Se(VI)}$ and $C_{Se(IV)}$ are selenate and selenite concentration; A_1 and A_2 are the absorbance signal in pH 2.0 and 8.8; m_1, m_2 and m_3 are the angular coefficients of selenate curve in an acid solution and selenate and selenite in alkaline solution, respectively; a_1, a_2 and a_3 are linear coefficients of selenate curve of in acid solution and selenate and selenite curve in alkaline solution, respectively;

As can be seen, this system of equations takes into account the different absorptivity of studied species at different pH values.

The calibration equations were: A = 0.140 $C_{Se(IV)}$+0.0142, r = 0.9996; A = 0.158 $C_{Se(VI)}$+ 0.0506, r = 0.9992 and A = 0.154 $C_{Se(VI)}$+0.0677, r = 0.9985, respectively for selenite and selenate in pH 8.8 and selenate in pH 2.0, where A is the absorbance and r is the linear correlation coefficient.

3.4 Study of concomitants

The studied concomitants (see experimental section) were added to a solution of 3 µg L^{-1} selenite and 2 µg L^{-1} selenate. The chloride and nitrite ions caused interference only when they were in the maximum permitted concentration allowed by CONAMA [21], but such situation rarely occurs in analysis of natural water samples. The others ions tested did not show interference in selenium analysis.

3.5 Figures of Merit of speciation system

The precision was evaluated in two levels: repeatability [26] (intra-run precision) and intermediary precision (inter-runs precision). It was obtained by preconcentrating (n=6) standard selenite and selenate solutions at 2.0 µg L^{-1}. Each solution was adjusted in the two studied values of pH (2.0 and 8.8) and submitted to the determination. The relative standard deviations obtained for selenate and selenite are always lower than 7.8 % and 7.3%, respectively.

The linearity was determined by the correlation coefficient (r), limits of detection and limits of quantification of the speciation system. They were based on 3 and 10 times the blank standard deviation [27]. The data for selenate were obtained from the analytic curve built in acid medium. Table 3 shows the parameters of linear regression obtained for both selenium species.

Table 3 – Analytical parameters obtained from the analytical curves of Se (IV), pH 8.8 and Se (VI), pH 2.0; employing the preconcentration system for speciation of selenium.

Parameters	Analytical curve of Se (VI)	Analytical curve of Se (IV)
Linear range (µg L^{-1})	0.5 to 8	0.5 to 10
Linear coefficient	6.88x10^{-2}	1.42x10^{-2}
Angular coefficient (m) (L μg^{-1})	1.54x10^{-1}	1.40x10^{-1}
Correlation coefficient	0.9985	0.9996
LOD[a] (µg L^{-1})	3.1x10^{-2}	4.9x10^{-2}
LOQ[b] (µg L^{-1})	1.0x10^{-1}	1.6x10^{-1}

[a]LOD = (3 σ/m) [b]LOQ = (10 σ/m)

In order to evaluate the performance of preconcentration system, some parameters were obtained. The enhancement on sensitivity was determined by enrichment factor (EF) [12]. This factor is calculated by ratio of angular coefficients of analytical curves obtained before and after analyte preconcentration. The concentration efficiency reflects the sample frequency of the system. This parameter value indicates the EF obtained during one minute of preconcentration, and it is defined as the product of EF by the frequency of sampling (number of samples analyzed per minute). Other parameter evaluated was the consumption index (CI). It is based on sample loading required by preconcentration system. It is defined by the volume of sample (mL) consumed to obtain one unity of EF. This index is obtained by the ratio between the pre-concentrated sample volume and the enrichment factor of the system.

The evaluated parameters are shown in Table 4, where it is also shown a comparison between the present work and three published works that use an on-line ET-AAS pre-concentration system to speciate selenium [16-18]. This Table shows that the proposed system is efficient due to present similar or better parameters than those published, and in our work the sample preparation is easier because there is no reduction step. In the best of our knowledge, the principle of additivity of absorbance associated with atomic absorption measurements in speciation studies is here used for the first time.

Table 4 – Evaluated parameters related to efficiency of pre-concentration system in-line to ET-AAS for selenium speciation.

Characters	Proposed Method		References		
	Selenite	Selenate	[19]	[20]	[21]
Enrichment Factor (EF)	108	74	22	112	82
Consumption index (mL)	0.056	0.081	0.046	0.038	0.11
Concentration Efficiency (CE min^{-1})	37.0	25.4	14.7	20.53	8.2

The accuracy of the proposed method was checked from analysis of reference material (Dogfish). The obtained data were compared with the certified value by t-Test, where $T_{calculated}$ 0.4850 < T_{table} 4.303. Therefore, there were no statistic differences between the data in a confidence level of 95 %.

3.6 Application in real samples

Natural water samples and deionized water were spiked with different concentration of selenite and selenate, and analyzed. The results (Table 5) showed an excellent recovery of both species, however only selenite was found in one of the water samples collected in a region close to the Universidade Federal de Alfenas.

Table 5 – Selenium determination in spiked water sample.

Sample	Enrichment (µg L^{-1})		Determined value [a] (µg L^{-1})		Recovery (%)	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
Deionized water	6.0	3.0	6.4±0.1	2.9±0.07	107	97
	5.0	2.0	5.1±0.08	2.0±0.07	102	100
Natural water	3.0		2.2±0.03	ND	105	
			5.5 ± 0.05	ND		
	1.0	5.0	ND	ND	99	104
			1.0±0.009	5.2±0.01		
River 1[b]	3.0	3.0	ND	ND	99	109
			3.0±0.07	3.3±0.05		
River 2[c]	5.0	1.0	ND	ND	110	109
			5.5±0.08	1.1±0.07		

ND: not detected; [a] media ± relative standard deviation (n = 3)
[b] 21º26'54"S and 45º37'38"W; [c] 21º25'13"S and 46º01'34"W

The proposed method was employed in two samples of pharmaceuticals. The results for selenite was (5.00±0.08 and 5.00±0.009 mg g^{-1}) and selenate was not detected, these values are in agreement with the labeled by manufacturer.

4. Conclusion

The presented system shows some advantages as regard previous published methods for selenium speciation. It was showed that the use of two different pH values for the samples associated with the principle of absorbance additivity, avoid the commonly procedures based on reduction step. Moreover, the preconcentration/speciation method presents excellent analytical based on EF, CI and EC obtained.

The method presented satisfactory selectivity, since the more severe interferences were observed only for higher concentrations of Cl^- and NO_3^-.

There was no significant difference in 95% of confidence level between the proposed method and the certified value of the reference material (Dogfish liver). Recovery tests in natural water samples showed satisfactory results, ranging from 99 up to 110%, thus attesting the feasibility of the method for these kinds of samples. Finally, the relative standard deviation obtained with the intra-run and inter-runs always smaller than 7.9 % can be considered adequate.

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