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Hollow fiber liquid phase microextraction followed by high performance liquid chromatography for determination of ultra-trace levels of Se(IV) after derivatization in urine, plasma and natural water samples

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ABSTRACT

In the present work, a simple and high sensitive method based on hollow fiber liquid phase microextraction (HF-LPME) was developed followed by high performance liquid chromatography (HPLC) for determination of ultra-trace amounts of Se(IV) after derivatization in biological and natural water samples. Se(IV) was complexed with *o*-phenylenediamine to form piazselenol. The formed piazselenol was extracted into 20 μL of 1-octanol located in the lumen of a hollow fiber and the solution was injected into HPLC-UV for analysis. Using the Taguchi method, an orthogonal array design (OAD), OA_{16} (4^5) was employed to optimize the HF-LPME of piazselenol. The effect of five experimental factors (each factor at four levels) including the volume of the organic phase, extraction time, pH of the solution, stirring rate and ionic strength on the extraction efficiency of piazselenol was studied and optimized. The maximum extraction efficiency of piazselenol was obtained at 20 μL of 1-octanol as the extracting solvent, 30 min extraction time, pH 2, stirring rate of 500 rpm and 30% (w/v) NaCl. Under the optimum conditions, preconcentration factors up to 130 were achieved and the relative standard deviation (%RSD) of the method was <3.7% for different concentrations of Se(IV). The calibration curves were obtained in the ranges of 0.2–100 and 0.05–10 μg L^{-1} for the 11 and 50 mL of the sample volumes with reasonable linearity, respectively ($r^2 > 0.995$). The limits of detection (LOD) were 0.1 and 0.02 μg L^{-1} for the 11 and 50 mL sample volumes, respectively ($S/N = 3$). Finally, the applicability of the proposed method was evaluated by the extraction and determination of Se(IV) in the plasma, urine and water samples.

1. Introduction

Selenium, as one of the biologically essential elements for man, is found in aquatic systems with the concentrations ranging from less than 2 ng L^{-1} to thousands of mg L^{-1} [1]. The human nutritional requirement for selenium has been determined to be in the range of 0.1–0.3 mg kg^{-1} of diet. However, in the range of 2–10 mg kg^{-1} of diet, it produces some chronic toxicity symptoms as liver carcinoma, cirrhosis, teeth, hair and nail losses, irritation of the eyes and paralysis [2]. Although the median lethal dose of selenium varies with the kind of animals, the dose of sodium selenite in rats has been reported as 7 mg kg^{-1} [3], while that of sodium selenate is 31.5 mg kg^{-1} [4]. Because of the wide range of biologically relevant selenium concentrations and the many chemical forms of selenium, determination of selenium in biological samples has been problematic. A variety of analytical techniques such as UV–Vis spectrophotometry, spectrofluorimetry, voltammetry, atomic absorption spectrometry (AAS) and ion chromatography equipped with graphite furnace atomic absorption spectrometry (GF-AAS) or inductively coupled plasma mass spectrometry (ICP-MS) has been applied to determine selenium [5–15]. Some of the applied methods such as UV–Vis spectrophotometry and spectrofluorimetry have matrix spectral interference problems. Hydride generation atomic absorption spectrometry (HG-AAS) is the most sensitive AAS technique for selenium determination and has been extensively applied to biological materials [16]. The sensitivity and accuracy of GF-AAS is adequate for selenium determination in biological samples and the method requires little sample preparation. However, GF-AAS is very sensitive to matrix interferences [16]. Several reports based on high performance liquid chromatography (HPLC) [17], gas chromatography (GC) [18] and capillary electrophoresis (CE) [19] coupled with ICP-MS for selenium analysis have been published. Indeed, selenium detection using ICP-MS suffers from two main difficulties. The first one is linked to the high first ionization potential (9.75 eV) of

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this element, responsible of low ionization in argon (Ar) plasma. Secondly, as the most abundant selenium isotope, ^{80}Se (49.6%) is interfered by $^{40}Ar^{40}Ar^{+}$ dimer, a less interfered but also less abundant selenium isotope (^{82}Se, 9.2%) is commonly monitored. The development of ICP-MS equipped with collision/reaction cell (C/RC) has offered an interesting solution to overcome polyatomic interferences. However, there are some publications for determination of selenium species applying ICP-MS without C/RC system [20,21]. Further, ICP-MS is expensive and its maintenance is difficult [12].

Because of the low concentration of selenium and the complexity of the environmental samples, an enrichment step is usually needed prior to the instrumental analysis. Hydride generation [15], liquid–liquid extraction (LLE) [22,23] and solid-phase extraction (SPE) [24,25] are the most commonly used techniques for separation or preconcentration of selenium in the environmental and biological samples. However, for extraction or elution, these steps often require an appreciable amount of toxic solvents or reagents, which are hazardous to the operators and result in threat to the environment. Therefore, a variety of microextraction techniques that use no or small amounts of solvent have been developed in the recent years. Recently, liquid-phase microextraction (LPME) was developed as a fast, simple and inexpensive solvent-minimized liquid–liquid extraction technique [26,27]. Subsequently, hollow fiber liquid phase microextraction (HF-LPME), based on the application of a supported liquid membrane was introduced [28,29]. In HF-LPME, the organic solvent is injected into the lumen of the porous hollow fiber, and the fiber acts as an interface between the sample solution and the extracting phase. Since very little amounts of the solvent are used, exposure of the operator to toxic organic solvents is minimized. At the same time, the technique combines extraction, concentration and sample introduction into one step. It has been successfully used for the determination of chemical warfare agents [30], dichlorophenol isomers [31], heterocyclic aromatic amines [32], short-chain fatty acids [33], insecticides [34], endocrine disrupting alkylphenols, chlorophenols and bisphenol-A [35], some metal ions [36,37] and so on. Recently, Xia et al. published their results for Se speciation in natural water samples by cycle-flow SDME and HF-LPME combined with electrothermal vaporization (ETV)-ICP-MS [38]. They used ammonium pyrrolidine dithiocarbamate (APDC) as a chelating reagent both for the microextraction and the chemical modifier for Se determination. However, APDC is not a selective ligand for Se(IV) and there are many publications based on using this ligand for the extraction of different metal ions. Also, ETV-ICP-MS is very expensive and its maintenance is very difficult.

The fluorometric method, based on the reaction of Se(IV) with aromatic *o*-diamines (AODs) specially 2,3-diaminonaphthalene to form fluorescent naphtho-2-selena-1,3-diazole (4,5-benzopiazselenol) [39] has been the most widely used chemical method for the determination of trace levels of selenium in biological materials. One of the two absorption maxima of Se(IV) complexes with AODs occurs at around 330 nm and the other at around 430 nm. Since AODs absorb strongly at the wavelength of 330 nm but negligibly at 430 nm, thus absorbances are measured at the wavelength of 430 nm [40,41]. The molar absorptivity of piazselenol around the wavelength of 330 nm is strongly high in comparison with its value around 430 nm. Therefore, by separation of excess AODs from piazselenol using HPLC, detection at 330 nm is possible and a considerable enhancement in LOD is observed. In the present study, Se(IV) in the water and biological samples was reacted with *o*-phenylenediamine to form piazselenol (λ_{max} = 332 nm). The resulted piazselenol was extracted into 20 μL of 1-octanol located inside the hollow fiber and determined by HPLC-UV instrument. Taguchi method [42] was applied to optimize HF-LPME of the obtained piazselenol.

2. Experimental

2.1. Chemicals

Analytical-grade 1,2-diaminobenzene (*o*-phenylenediamine) from Fluka (Chemie AG, Switzerland) was used as received. HPLC-grade acetonitrile and methanol were purchased from Aldrich (Milwaukee, WI, USA). The 1000 mg L^{-1} stock standard solutions of Se(IV) were prepared by dissolving appropriate amounts of H_2SeO_3 (Merck, Darmstadt, Germany) in ultra-pure water. 1-Octanol was purchased from Merck (Darmstadt, Germany). *o*-Phenylenediamin solution in 0.1 M HCl was prepared daily. The water used in the experiments was purified on a Milli-Q ultra-pure water purification system purchased from Millipore (Milford, MA, USA). Urine sample was collected from a healthy volunteer and the sample preparation was performed immediately. Plasma sample was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran) and stored at −20 °C prior to use. All the other chemicals were of reagent grade or of the highest purity available. Plastic and glassware used for the experiments were previously soaked in 0.1 M nitric acid for 24 h and rinsed carefully with ultra-pure water.

2.2. Apparatus

Chromatographic separations were carried out on a Varian HPLC containing a 9012 HPLC pump (Mulgrave Victoria, Australia), a six-port Cheminert HPLC valve (Valco Instruments, Houston, USA) with a 20 μL sample loop and a Varian 9050 UV–Vis detector. Chromatographic data were recorded and analyzed using Chromana software (version 3.6.4). The separations were carried out on an ODS-3 column (100 mm × 4.0 mm, with 3 μm particle size) from MZ-Analitical Company (Germany). Mixture of 50 mM ammonium acetate, pH 4.6 (A solvent) and methanol (B solvent) was under gradient elution condition used as mobile phase. The gradient program was: 30–60% B in A, from 0 to 13 min; 60–90% B in A, 13–16 min; returned to initial conditions in 5 and 5 min of post-run delay. The injection volume was 20 μL for all the samples and the detection was performed at the wavelength of 332 nm. GF-AAS measurements were carried out by an atomic absorption spectrometer GBC, Avanta PM (Victoria, Australia) equipped with a graphite furnace atomizer GF 3000 and an autosampler (Pal 3000). Deuterium background correction was employed to correct non-specific absorbance. Peak height absorbance was chosen as the analytical signal. The instrumental parameters and temperature program for the graphite atomizer are listed in Table 1. A Cesil CE-7200 UV–Vis spectrophotometer (Cambridge, England) was applied for the absorbance measurements of the solutions. All of the pH measurements

Table 1
Instrumental parameters for determination of Se(IV) using GF-AAS.

Spectrometer parameter				
Wavelength (nm)		196.0		
Slit width (nm)		1.0		
Lamp current (mA)		10.0		
Step	Temperature (°C)	Time (s)		Gas flow (L min^{-1})
		Ramp	Hold	
Graphite atomizer				
Pre-warming	50	1.0	2.0	3.0
Inject step	Inject sample	–	–	3.0
Drying	120	15.0	10.0	3.0
Pyrolysis or ashing	900	10.0	5.0	3.0
Gas stop step	900	0.0	1.0	0.0
Atomization	2100	0.8	1.0	0.0
Cleaning	2300	1.0	2.0	3.0

were performed with a WTW Inolab pH meter (Weilheim, Germany).

2.3. Derivatization of Se(IV) in standards and real samples

Half millilitre of appropriate concentration of *o*-phenylendiamine in 0.1 M HCl was added to a 50 mL glass vial containing the sample solution (standard or real) whose pH was previously adjusted at 2 using 2 M HCl. In order to reduce the matrix effect, the plasma and urine samples were diluted (1:10) with ultra-pure water before the addition of *o*-phenylenediamine. The derivatization temperature and the needed time to form the corresponding piazselenol were selected based on our previous work [43] at 90 °C and 30 min, respectively. When derivatization was completed and the solution cooled down to room temperature, it was transferred into a 50-mL volumetric flask and diluted to the mark by the addition of ultra-pure water.

2.4. Extraction procedure

All of the extractions were carried out using a Q 3/2 Accurel polypropylene hollow fiber membrane (Membrana, Wuppertal, Germany) with a 0.2 μm pore size, 600 μm internal diameter and 200 μm wall thickness. The hollow fibers were cut into appropriate length pieces. Each fiber was used only once to decrease the memory effect. Before using, each hollow fiber was sonicated for 5 min in acetone to remove any possible contaminants. It was then removed from acetone and the solvent was allowed to evaporate completely. For each experiment, 11 mL of the aqueous sample containing piazselenol was poured into a 12 mL sample vial having a 4 mm × 14 mm magnetic stirring bar. The sample vial was placed on an IKA multi-station magnetic stirrer (Staufen, Germany) and a 25 μL Hamiltone microsyringe (Bondaduz, Switzerland) was applied to introduce the organic phase (1-octanol) into the hollow fiber. The hollow fiber was then immersed in the organic solvent for 10 s to impregnate its pores with organic phase. The organic solvent was introduced into the hollow fiber with slow pushing of the microsyringe plunger and then the fiber was inserted into the water for 10 s to wash extra organic solvent from the surface of the hollow fiber. Finally, the end of the hollow fiber was sealed by a piece of aluminum foil. The hollow fiber was introduced into the sample vial at U-shape configuration and the top of the vial was covered with a piece of Parafilm. The extraction was done during the prescribed period of time. Then, the microsyringe containing the hollow fiber was removed from the sample vial and the end of the hollow fiber was opened. Finally, the receiving phase was withdrawn into the microsyringe and injected into the 20 μL HPLC loop followed by analysis.

3. Results and discussion

Se(IV) reacts with AODs in acid medium to form yellow piazselenol, which is sparingly soluble in water and utilize for spectrophotometric determination of selenium [40]. In the present study, *o*-phenylenediamine was used to react with Se(IV) to form an extractable piazselenol with λ_{max} = 332 nm. Two-phase HF-LPME was applied for the extraction and perconcentration of piazselenol in the aqueous samples.

Taguchi method is a type of fractional factorial design in which orthogonal array (OA) is used to assign the selected factors to a series of experimental combinations, whose results can then be analyzed using a common mathematical procedure. Recently, several applications of this method have been reported [44,45]. A more detailed description of an orthogonal array design has been given elsewhere [46]. For a two- or three-level design, great care in choosing the levels of each factor is required (the level of each factor is defined as a value that the factor is adjusted to perform an experiment). However, for a four-level design, the selection process is much easier and less likely to lead to misleading results. Therefore, a four-level design results in a more detailed display of how each response function is affected by the changes in the variables. The results of orthogonal array design (OAD) experiments can be treated by the analysis of variance (ANOVA) and/or the direct observation analysis (also called range analysis) [42,47]. In ANOVA, the effects of different factors on the response function can be evaluated by computing *F*-ratio (variances ratio) and percent contribution (PC) values. Meanwhile, the effect of a factor or the interaction among different factors on the response function can also be evaluated using *F* and PC-values. In direct observation analysis, the responses versus the levels of different factors can be observed directly from a broken line plot. It is noteworthy that the factors most significantly affecting the output can be obtained from both ANOVA and direct observation analysis of the experimental data. The equations used to calculate the sum of squares, mean squares, degree of freedom and *F*-values in the ANOVA method have already been reported [42].

3.1. Extracting solvent

The selection of extracting solvent is of great importance in HF-LPME in order to obtain an efficient extraction. In the selection of the extracting solvent, three factors should be considered. First, the organic solvent must be compatible with the fiber so that the pores of the fiber can be filled completely. This is important since the extraction occurs on the surface of the solvent immobilized in the pores. Second, the organic solvent must be immiscible with water. Finally, the organic solvent should have good chromatographic behavior.

On the basis of these considerations, 1-octanol, 1-hexanol, methyl isobutyl ketone (MIBK) and benzyl alcohol were investigated in the preliminary experiments, in which 15 min extractions from 11 mL of 30 μg L^{-1} standard solutions (adjusted at pH 2 and stirred at 800 rpm) were carried out. The experiments indicated that the extractions were significantly higher in the presence of 1-octanol in comparison with the other solvents. Additionally, 1-octanol was easily immobilized on the pores of the fiber within a few seconds. Further, its solubility in water is low and its chromatographic behavior is suitable. Accordingly, 1-octanol was selected for the subsequent experiments.

3.2. Experimental design and data analysis

A five-factor four-level factorial design was applied to evaluate the effects of such a factors as volume of the organic phase (1-octanol), pH of the sample solution, stirring rate, ionic strength and extraction time on the HF-LPME of piazselenol. In order to estimate the best condition for the extraction of piazselenol, 16 experiments were performed. The factors and their respected levels are reported in Table 2. For increasing the precision of the optimization process, each trial was repeated twice (n = 32). The mean values of the peak areas for the corresponding factors and at each level was calculated according to the assignment of the experiment. For example, the peak areas of the four trials at pH 2 were evaluated as the mean value of the corresponding eight runs. The mean value of the four levels of each factor (e.g. pH) reveals that how the extraction will change when the level of that factor is changed. Fig. 1 shows the mean peak area as a function of the levels of the studied factors. According to the results (Fig. 1), the optimum values of the selected factors for the HF-LPME of piazselenol were 20 μL of 1-octanol, pH 2, stirring rate of 500 rpm, addition of 30% (w/v) NaCl and 30 min extraction time. ANOVA results for the calculated models are shown in Table 3. Comparison of the calculated *F* values of each factor with the corresponding critical values at the degrees of freedom

Table 2
OA_{16} (4^5) experimental design for the optimization of HF-LPME of Se(IV).

Trail no.	Extraction time (min)	%Salt (w/v)	Stirring rate (rpm)	pH	Organic phase volume (μL)
1	10	0	200	2	8
2	10	10	500	5	12
3	10	20	800	8	16
4	10	30	1000	10	20
5	20	0	500	8	20
6	20	10	200	10	16
7	20	20	1000	2	12
8	20	30	800	5	8
9	30	0	800	10	12
10	30	10	1000	8	8
11	30	20	200	5	20
12	30	30	500	2	16
13	45	0	1000	5	16
14	45	10	800	2	20
15	45	20	500	10	8
16	45	30	200	8	12

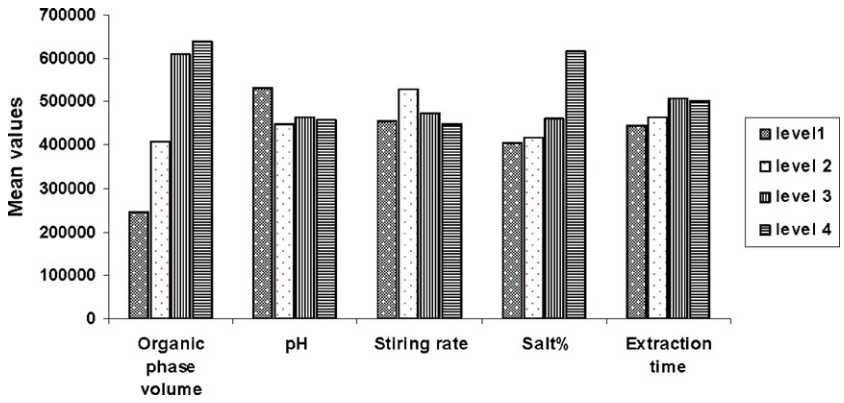

Fig. 1. The effect of the volume of the organic phase, pH of extraction, stirring rate, %salt and extraction time on the HF-LPME of Se(IV). Levels of the factors are: volume of the organic phase: 8, 12, 16 and 20 μL of 1-octanol; pH of extraction: 2, 5, 8 and 10; stirring rate: 200, 500, 800 and 1000 rpm; %salt: 0, 10, 20 and 30% (w/v) of NaCl; extraction time: 10, 20, 30 and 45 min.

equal to 3 (for each factor) and 16 (for error) and confidence level of 95% showed that all of the selected five factors had significant effect on the extraction efficiency of piazselenol. The volume of 1-octanol and the ionic strength had the highest effects on the extraction efficiency with the percent contributions of 71.2% and 20.8%, respectively. The other parameters including extraction pH, extraction time and stirring rate had the percent contributions of 2.9%, 1.6% and 2.8%, respectively.

3.2.1. Influence of ionic strength

For HF-LPME in aqueous solutions, the addition of salt (such as NaCl or Na_2SO_4) can decrease the solubility of the analytes and enhance their partitioning into the organic phase (salting-out effect). The optimization experiments were carried out by adding NaCl into the sample solution in the range of 0–30% (w/v). It was demonstrated that salt addition had remarkable effect on the extraction efficiency of piazselenol when the NaCl concentration increased from 0% to 30% (Fig. 1). Therefore, the extractions were carried out from the samples containing 30% NaCl.

3.2.2. Influence of the volume of the organic phase

The effect the volume of the organic phase on the extraction efficiency of piazselenol was also investigated at four levels in the range of 8–20 μL. As shown in Fig. 1, the extraction recovery increased by increasing of the organic phase volume due to the increase in the number of moles of the analyte transferred into the organic phase. Fig. 1 shows that in the presence of 20 μL organic solvent (1-octanol), maximum extraction efficiency was obtained.

Table 3
ANOVA results for the optimization of HF-LPME of Se(IV).

Factor	DOF[a]	Sum of squares	Variance	*F*-ratio[b]	Pure sum of squares	%PC[c]
Extraction time	3	185.436	61.812	21.446	176.789	1.57
%Salt	3	2309.655	769.885	267.117	2301.008	20.42
Stirring rate	3	323.610	107.870	37.426	314.964	2.79
pH	3	359.120	119.708	41.533	350.479	3.11
Organic phase volume	3	8045.948	2681.982	930.533	8037.302	71.32
Error	16	46.114	2.882			0.80
Total	31	11269.890				100

[a] Degrees of freedom.
[b] $F_{critical\,(3,16;0.05)} = 3.24$.
[c] Percent of contribution.

3.2.3. Influence of stirring rate on the extraction efficiency

Extraction kinetics can be accelerated by stirring of the samples. If the stirring rate is too high, solvent loss and formation of air bubbles may occur, which would affect the extraction efficiency. As shown in Fig. 1, by increasing of the stirring rate from 200 to 500 rpm, the extraction efficiency of piazselenol increased but at higher stirring rates (800 and 1000 rpm), due to the formation of air bubbles in the extraction vial and surrounding of the hollow fiber by air bubbles, the extraction efficiency of piazselenol decreased. Accordingly, the stirring rate of 500 rpm was selected as the optimum value for the subsequent experiments.

3.2.4. Influence of extraction time

The effect of extraction time on the extraction of piazselenol is shown in Fig. 1. One can see that by increasing of the extraction time from 10 to 30 min, the extraction recovery increases. A small decrease in the extraction recovery at higher extraction times (45 min) is due to a slight losing of the organic solvent that occurs at longer extraction times. Therefore, 30 min was selected as the optimum extraction time for the subsequent experiments.

3.2.5. Influence of extraction pH

The reaction between Se(IV) and *o*-phenylenediamine was performed at pH 2. Once the complexation reaction was completed, to investigate the effect of pH on the extraction of piazselenol, pHs of the samples were adjusted at 2, 5, 8 and 10 by the addition of 1 M NaOH, according to the experimental design (Fig. 1). The results showed that the maximum extraction efficiency of piazselenol was obtained at pH 2. At higher pH values, the extraction efficiency decreased because the suitable range of pH for complexation of Se(IV) with AODs is 2–3 [40]. Also, at higher pHs, the resulted piazselenol might be decomposed. Therefore, the extraction pH 2 was selected as the optimum value.

Further experiments were performed under the optimum conditions. The obtained results showed that the recoveries were similar to the optimum performance calculated using the expression:

$$A_{opt} = \frac{T}{N} + \left(\bar{v} - \frac{T}{N}\right) + \left(\bar{s} - \frac{T}{N}\right) + \left(\bar{p} - \frac{T}{N}\right) + \left(\bar{r} - \frac{T}{N}\right) + \left(\bar{t} - \frac{T}{N}\right)$$

where T is the grand total of all results, N is the total number of results, A_{opt} is the performance under the optimum conditions and $\bar{v}$, $\bar{s}$, $\bar{p}$, $\bar{r}$ and $\bar{t}$ are the average performances of the volume of 1-octanol, %salt, pH, stirring rate and extraction time at their optimum levels. Based on the above equation, under the optimum conditions, the performance is estimated using only the significant factors (all the factors in this study) [40].

Under the optimum conditions, the confidence interval (C.I.) of the performance is calculated using the following expression:

$$\text{C.I.} = \pm\sqrt{\frac{F(1, n_2) \times V_e}{N_e}}$$

where $F(1, n_2)$ is the F-value from the F-Table at a required confidence level at the degrees of freedom of 1 and of error, n_2; V_e is the variance of error term (from ANOVA) and N_e is the effective number of replications. Taguchi method predicted that the results under the optimum conditions have to be in the range of 902,849–938,053 (based on the peak areas). The average of results of the experiments ($n = 3$) under the optimum conditions was 907,270 ± 19,231, showing that Taguchi optimization method can be a rapid and safe method for optimization of HF-LPME method.

3.3. Interference study

The reaction of Se(IV) with *o*-phenylenediamine is almost specific. Tellurium does not react with AODs, but some metal ions such as Fe^{3+} and Cu^{2+} can oxidize the ligand, giving colored oxidation products [40]. The influence of several ions present in the natural waters and biological samples on complexation and extraction of 1.0 μg L^{-1} of Se(IV) was investigated under the optimum conditions. The concentration ranges of these ions were chosen in view of their maximum levels in the natural water and biological samples (Table 4). The results showed that the presence of 1000 μg L^{-1} of Al^{3+}, As(III), Cd^{2+}, Co^{3+}, Cr^{3+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} had no significant effect (recovery between 90% and 110%) on the complexation and HF-LPME of Se(IV). Only Cu^{2+} was found to significantly interfere at the concentrations higher than 1000 μg L^{-1}, but such levels are not likely to be found in the natural waters. However, in the case of high concentrations of Cu^{2+}, the interference effect could be minimized by the addition of 1 mL of 5% EDTA solution as the masking agent. Na^+ up to 10,000 mg L^{-1} and Ca^{2+}, Mg^{2+}, K^+ up to 500 mg L^{-1} did not cause any significant interference. Finally, a number of common anions like Cl^-, F^-, PO_4^{3-} and CO_3^{2-} were tested and no interference was found at the concentrations up to 15,000 mg L^{-1} for Cl^- and 50 mg L^{-1} for the other anions.

Table 4
Effect of foreign ions on the HF-LPME of Se(IV).

Foreign ions	Concentration of ion (mg L^{-1})[a]	%Recovery
As(III)	1.0	103 ± 4
Al^{3+}	1.0	104 ± 6
Ca^{2+}	50.0	93 ± 5
Cd^{2+}	1.0	98 ± 4
Co^{3+}	1.0	99 ± 4
Cr^{3+}	1.0	108 ± 2
Cu^{2+}	1.0[b]	104 ± 5
Fe^{3+}	1.0	94 ± 1
K^+	50.0	94 ± 2
Mg^{2+}	50.0	96 ± 1
Mn^{2+}	1.0	99 ± 4
Na^+	10,000	111 ± 7
Ni^{2+}	1.0	100 ± 5
Pb^{2+}	1.0	90 ± 10
Zn^{2+}	1.0	89 ± 5
CO_3^{2-}	50.0	105 ± 6
F^-	1.0	103 ± 6
PO_4^{3-}	50.0	103 ± 6
Cl^-	15,000	111 ± 7

[a] In the presence of 1 μg L^{-1} Se(IV).
[b] The interference was masked by the addition of 1 mL of 5% EDTA solution.

3.4. Method validation

3.4.1. Analytical performance

The figures of merit of the proposed HF-LPME method including the preconcentration factor, dynamic linear range (DLR), limit of detection (LOD) and percent of extraction were investigated for the extraction of Se(IV) from the aqueous solutions under the optimum conditions. The results are summarized in Table 5. Two calibration curves were plotted using ten spiking levels of Se(IV) in the concentrations ranged from 0.2 to 100 μg L^{-1} (sample volume = 11 mL) and from 0.05 to 10 μg L^{-1} (sample volume = 50 mL). For each level, three replicate extractions were performed under the optimum conditions. LOD values based on the signal to noise ratio of three were 0.1 and 0.02 μg L^{-1} for the 11 and 50 mL sample solutions, respectively. Preconcentration factor and percent of extraction for the 11 mL sample were 50 times and 11% and for 50 mL sample, they were 130 times and 6%, respectively.

Comparison of the proposed method with the other methods (LLE, HG, SPE, etc.) for the extraction and determination of Se(IV) is shown in Table 7. The extraction method, applied in the present work (HF-LPME), has some advantages in comparison with the other extraction methods including; low consumption

Table 5
Figures of merit for the HF-LPME of Se(IV)[a].

Preconcentration factor	50[b] 130[c]
DLR	0.2–100[b] 0.05–10[c]
LOD	0.1[b] 0.02[c]
Calibration equation	$A = 5936\,C + 3100$[b] $A = 9539\,C + 81$[c]
Percent of extraction	11%[b] 6%[c]
Repeatability ($n = 3$)	<3.7%
Reproducibility ($n = 6$)	7.1%

[a] All concentrations are in $\mu g\,L^{-1}$.
[b] For 11 mL sample.
[c] For 50 mL sample.

of organic solvents and reagents, simplicity and low cost of the extraction device, minimum carryover and cross-contamination and producing a clean extracting phase for the analysis. Detection limit and linear range of the proposed method are better than those of the methods that used UV–Vis spectrometry, GF-AAS, differential pulse polarography (DPP) and gas chromatography-flame ionization detector (GC-FID), and are comparable with those of the methods that used ICP-MS as detection system. Also the precision (%RSD) of this method is comparable with that of the other methods (Table 7).

3.4.2. Extraction of Se(IV) from water, plasma and urine samples

In order to study the suitability of the proposed HF-LPME method for the extraction and determination of Se(IV) in the real samples, the developed technique was applied for the extraction of Se(IV) from the tap water (Tehran, Iran), well water (Laleh Park, Tehran, Iran), plasma and urine samples. Standard addition method was applied for the measurement of Se(IV) in all of the real samples. The concentration of Se(IV) in the tap and well water samples were found as 0.20 ± 0.01 and $0.12 \pm 0.01\ \mu g\,L^{-1}$, respectively (Table 6). The results are in agreement with those reported in the literature [22,48–50].

Table 6
Comparison between the results of HF-LPME-HPLC-UV and LLE-GF-AAS methods for determination of Se(IV) in the tap water, well water, plasma and urine samples.

Sample	Concentration ($\mu g\,L^{-1}$)	
	HF-LPME-HPLC-UV	LLE-GF-AAS
Tap water	0.20 ± 0.01	–
Well water	0.12 ± 0.01	–
Plasma	46 ± 4	45 ± 5
Urine	19 ± 3	23 ± 4

In order to reduce the matrix effect, the urine and plasma samples were diluted to 1:10 using ultra-pure water. The derivatization and the extraction steps were performed according to Sections 2.3 and 2.4. The concentration of Se(IV) in the plasma and urine samples was determined as 46 ± 4 and $19 \pm 3\ \mu g\,L^{-1}$, respectively (Table 6). The chromatograms of analysis of 11 mL diluted non-spiked and spiked ($10\ \mu g\,L^{-1}$) plasma samples with Se(IV) are shown in Fig. 2. Several peaks appeared in the chromatograms are related to the compounds presented in the plasma samples. It is clear that in two-phase LLE methods, hydrophobic compounds can be extracted into the organic phase and the peaks appeared in the chromatograms are related to those that have appropriate absorption at $\lambda = 332$ nm. The assignment of these peaks is out of the scope of the current study. Finally, in order to investigate the accuracy of the proposed method, Se(IV) content of the real samples was extracted using LLE method. The method was based on the derivatization of Se(IV) with *o*-phenylenediamine and liquid–liquid extraction using toluene. The extraction procedure was conducted according to the standard method described in Ref. [40], but, determination of the extracted derivative was performed using GF-AAS instead of UV–Vis spectrophotometry (Table 6). One can see that a satisfactory agreement exists between the results obtained for the Se(IV) in the real samples by our proposed method and the GF-AAS.

Table 7
Comparison of the proposed method with the other methods for determination of Se(IV).

Method of extraction	Detection system	DLR ($\mu g\,L^{-1}$)	LOD ($\mu g\,L^{-1}$)	%RSD	Ref.
LLE[a]	GC–MS[g]	n.a.	1 ng	5.0	[22]
HG[b]	ICP-MS[h]	n.a.	0.03 (500 mL)	4.2	[51]
–	DPP[i]	3.9–530.4	n.a.	5.5	[52]
SPE[c]	GF-AAS[j]	n.a.	0.049 (100 mL)	4.3	[24]
FI-HG[d]	IC–ICP-MS[k]	0–10	0.04 ng	2.1	[20]
LLE	UV–Vis[l]	1000–7000	166.5	n.a	[23]
LPME[e]	GC-FID[m]	20–1000	0.9	3.2	[43]
HF-LPME[f]	ETV[n]–ICP-MS	0.005–40	0.0005	7.1	[38]
HF-LPME	HPLC-UV[o]	0.2 0.05	0.1 (11 mL) 0.02 (50 mL)	<3.7	The proposed method

[a] Liquid–liquid extraction.
[b] Hydride generation.
[c] Solid phase extraction.
[d] Flow injection hydride generation.
[e] Liquid phase microextraction.
[f] Hollow fiber liquid phase microextraction.
[g] Gas chromatography–mass spectrometry.
[h] Inductively coupled plasma mass spectrometry.
[i] Differential pulse polarography.
[j] Graphite furnace atomic absorption spectrometry.
[k] Ion chromatography inductively coupled plasma mass spectrometry.
[l] UV–Vis spectrophotometry.
[m] Gas chromatography with flame ionization detector.
[n] Electrothermal vaporization.
[o] High performance liquid chromatography with UV detector; n.a: data not available.

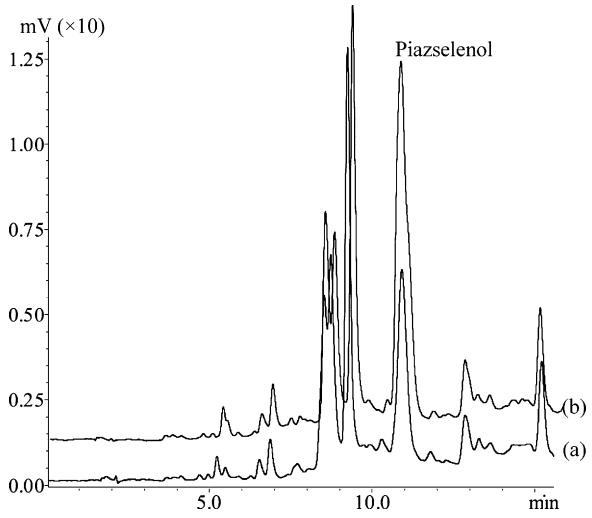

Fig. 2. HPLC chromatograms of the diluted (1:10) plasma samples for (a) non-spiked, (b) 10 μg L^{-1} Se(IV) spiked.

4. Conclusion

The main goal of this study was to develop a simple and sensitive method for the extraction and determination of ultra-trace amounts of Se(IV) in the natural water and biological samples. This method is based on the reaction of Se(IV) with *o*-phenylenediamine and extraction of the resulted piazselenol using HF-LPME followed by HPLC-UV analysis. Due to the simplicity and low cost of the extraction device, the hollow fiber can be discarded after each extraction to avoid carryover and cross-contamination. This serves to maintain high reproducibility and repeatability. In this paper, the excellent clean up property of the porous hollow fiber was applied to extract and determine Se in very complex matrices such as urine and plasma samples. Also in the present study, an orthogonal array design (Taguchi method) was employed to optimize HF-LPME of Se(IV). Based on the obtained results, a precise optimum condition with minimum number of experiments was achieved. It can be concluded that this new approach, based on the combination of three features of using a selective reagent with high molar absorptivity, using two phase HF-LPME to produce a clean and concentrated organic phase and applying HPLC system to separate piazselenol from the other UV-absorptive species including matrix components, leads to a simple, cheap, fast and high efficient determination of ng L^{-1} amounts of Se(IV) (DLR: 50–10,000 ng L^{-1}, LOD: 20 ng L^{-1}) in the natural water and biological samples, thus it is applicable for clinical and chemical laboratories.

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