Direct Determination of Selenium in Serum Matrix by Electrothermal Atomic Absorption Spectrometry: Application on Healthy Individuals from Algeria

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Abstract: Selenium is a trace element implicated significantly in oxidative stress in biological systems. In this work, a direct method for selenium determination in serum samples by electrothermal atomic absorption spectrometry with Zeeman background correction is proposed.

Serum samples were five-fold diluted in a 0.14% HNO_3 and 0.2% Triton X-100 **s**olution. Aliquots of 20 µL of the diluted serum samples were directly introduced into transversely heated graphite tubes. A total of 5 µg Pd and 3 µg $Mg(NO_3)_2$ was used as chemical modifier. Optimization of heating program was conducted by varying pyrolysis and atomization characteristic values. The optimal conditions were found to be 1400 and 2000 °C for pyrolysis and atomization temperatures respectively and 11 seconds for pyrolysis hold time.

The standard additions method was employed for calibration. Intra-day and inter-day validation using quality control samples at each point of the addition calibration curve were performed. Good accuracy, precision and recovery were achieved with the proposed method (less than 5% for accuracy and precision). A characteristic mass of 92.550 pg, a limit of detection of 4.010 ppb and a limit of quantification of 13.375 ppb in undiluted serum samples were obtained.

Several reference values of serum selenium concentrations were reported. As an application of the present method, selenium levels were determined in serum samples collected from 20 healthy individuals from Northern-Center of Algeria. Selenium concentrations ranged from 86.883 to 133.955 ppb with a mean value of 110.824±11.898 ppb.

Keywords: Selenium, serum, optimization, electhrothermal atomic absorption spectrometry, healthy individuals.

1. INTRODUCTION

Selenium is an essential trace element for many mammalian species, including humans. The content of selenium in human body varies from 6 to 20 mg, approximately half of it is distributed in muscle tissues [1, 2]. Serum levels of selenium in general population is usually ranged from 75 to 120 ppb. However, wide individual variations was reported [3, 4].

Concerning the physiological action, selenium is an essential constituent of various enzymes with antioxidant activity. This element is located at the active sites of selenoproteins where it is necessary for its activities. Selenoproteins play a key role in protecting cell components against free radical attacks. This function is mainly exerted by glutathione peroxidase (GPx) and thioredoxin reductase (TR). GPx detoxifies hydrogen peroxide and organic hydroperoxides to harmless products (water and alcohols). TR plays an important role in antioxidant system by breaking intramolecular disulfide bridges and regenerating the reduced form of antioxidant compounds such as vitamins C and E. Therefore, low levels of selenium in biological system may alter antioxidant system and then lead to pathological situations associated with oxidant stress like cancer and cardiovascular diseases [1]. Other biological activities have been attributed to this trace element. It has been identified as an integral part of iodothyronine 5'-Deiodinase which plays a key role in thyroid hormone metabolism. In addition, selenium is of great importance in anti-inflammatory, spermatozoa development and chemo-preventive processes [5-7].

The recommended daily amount of selenium is 40-55 µg (Food and Nutrition Board- Recommended dietary allowances, 2000). Dietary deficiencies are usually attributed to its low content in food, which in turn depends on its content in soil in which is found as

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ISSN: 2223-3806 / E-ISSN: 1927-5951/18

mineral (selenite and selenate) or (selenocystine and selenomethionine) organic compounds [4, 8-12].

Selenium deficiency can lead to Keshan disease with cardiomyopathy and Kaschin-Beck disease with osteoarthropathy. Moreover, arthritis, arterosclerosis, cataract, muscular dystrophy, sexual dysfunction, infectious disease**,** haematological abnormalities (anemia and erythrocyte macrocytosis) and aging have been associated with selenium deficiency. All these pathologic changes may be prevented and treated with selenium supplementation.

On the other hand, selenium can be toxic at high levels and leads to gastrointestinal, ocular and respiratory disorders, hair and nail loss, skin lesions and nervous system abnormalities.

For these reasons, determination of selenium in body fluids with an accurate method is useful in deficiency and poisoning situations.

Electrothermal atomic absorption spectrometry (ETAAS) is a very sensitive and a low sample requirement technique. It has been widely used for selenium determination in various clinical samples. Spectral interferences caused by iron and phosphorus species and low temperature losses are the main issues encountered in selenium quantification in biological tissues and fluids by ETAAS. Chemical modifiers, heating program optimization and Zeeman effect background correction understate such problems.

The aim of this study was the development and optimization of a rapid and sensitive method for selenium determination in serum samples by ETAAS. This method offers short analysis time which is convenient in poisoning situations.

Direct determination of selenium in sera was performed in order to prevent the loss of this trace element during pretreatment steps and to develop a simple method for large scale analysis.

Palladium and magnesium nitrate as chemical modifiers are known to be convenient for selenium determination in biological matrices. Therefore, this chemical modifiers were used in the proposed method. Heating program was optimized in order to minimize matrix interferences and to reach higher specific signals.

This method was successfully applied for selenium determination in serum samples from healthy individuals.

2. MATERIALS AND METHODS

2.1. Chemicals

High-purity water for both preparative and cleaning purpose was produced by double distillation for ultrapure deionized water (18.2 MΩ-cm, type I) by bidistillation apparatus (PureLab Option-Q).

All chemicals used were of analytical reagent grade.

A 1000 ppm selenium solution (Panreac) was used for reference standard solution preparation.

A 0.65% palladium nitrate and a 2.11% magnesium nitrate solutions (Chem-Lab) were used for the preparation of the chemical modifier solution.

Triton X-100 (Panreac) was used as surfactant and viscosity modifier of serum samples. Nitric acid (69%, Panreac) was used for the preparation and stabilization of standard solutions and for sample pre-treatments.

All solutions were prepared and stored in decontaminated polypropylene bottles. All these polypropylene bottles were cleaned with an adequate detergent, soaked in 10% (v/v) nitric acid for 24 h, and rinsed with high-purity water.

2.2. Instrument

Measurements were carried out using a PerkinElmer AA 800 electrothermal atomic absorption spectrometer equipped with a longitudinal Zeeman-effect background correction, a transversely heated graphite furnace atomizer and an AS-800 autosampler (Perkin-Elmer). The spectrometer was operated by a Perkin-Elmer selenium electrodeless discharge lamp (EDL-System 2) at 196.0 nm. THGA tubes were obtained from Perkin-Elmer.

Argon (Linde) was used as purge gas, with 250 ml/min of flow rate during all heating cycle except the atomization step.

Data acquisition and processing were achieved using WinLab32 for AA software (Perkin-Elmer).

2.3. Sample Pre-Treatment and Standard Preparations

In this work, standard additions method was employed for selenium determination in serum matrix. The stock solution (10 ppm) were prepared by diluting a 1000 ppm selenium solution with 0.5% nitric acid.

For the preparation of the reference standard solution (100 ppb), the stock solution was properly diluted by a 0.14% HNO_3 and 0.2% Triton X-100 solution.

The addition standard solutions (10, 20, 30, 40, 50, 60 ppb) were prepared in autosampler vials by adding different volumes of the reference standard solution (100, 200, 300, 400, 500, 600 µl) to 200µl of serum. The resulting standards were completed by a 0.14% HNO_3 and 0.2% Triton X-100 solution to final volume of 1 ml.

Blood samples were collected from volunteers with no known illness. The samples were centrifuged at 5000 rpm for 5 minutes and the resulting sera were transferred to cleaned tubes and kept at 4°C until analysis. All serum samples were transferred into autosampler vials and five-fold diluted before analysis.

For all standard solutions and sample preparations, the micropipette was pumped several times in order to ensure proper homogenization.

2.4. ETAAS Procedure

Aliquots of 20 µl of diluted serum samples with 10 µl of the chemical modifier (Pd: 5 µg, $Mg(NO_3)_2$: 3 µg) were injected into graphite tubes. The instrumental operating conditions and optimization of the heating program are summarized in Table **1**.

Integrated absorbance (peak area) and peak height values were used as signal and peak aspect optimization parameters.

2.5. Validation Experiments

The proposed method was validated for recovery, sensitivity and linearity. In addition, an intra and inter-day validation was performed to evaluate the accuracy and precision of the measurements. All these validation experiments were carried out to allow a bio-analytical application of the present method.

3. RESULTS AND DISCUSSION

3.1. Optimization of Heating Program

Development of reliable method for selenium determination in biological matrices by ETASS is faced by some challenges, among them: Selection of chemical modifier and optimization of furnace heating program.

The selection of palladium and magnesium nitrate (Pd: 5 µg, $Mg(NO_3)_2$: 3 µg**)** as the most efficient chemical modifier was based on several studies which recommend this mixture for selenium stabilization in serum matrix during the heating process [13-17].

The mechanism of action of selenium thermal stabilization is based on Se-Pd interaction which avoid selenium volatilization losses from biological samples while matrix compounds are eliminated. For this reason, palladium is called analyte modifier. This action is potentialised by matrix modifiers like magnesium nitrate which improve matrix calcination.

Spectral interferences due to iron and phosphorus species in the analytical line (196.0 nm) were minimized by using Zeeman-effect background

Table 1: Instrumental Settings and Heating Program

Parameters	Values
Current lamp (mA)	2.0
Wavelength (nm)	196
Background correction	Zeeman
Sample injection volume (µl)	20
Modifier injection volume (µl)	10
Matrix modifier	Pd: 5 µg
	$Mg(NO_3)_2$: 3 µg

tinging program	Temperature (°C)	Ramp time (s)	Hold time (s)	Gas flow (ml/minute)
Dry 1	110	1	30	250
Dry 2	130	15	30	250
Pyrolysis	1000-1500	10	6-31	250
Atomization	1700-2000	0	3	0
Cleaning	2600	1	5	250

correction integrated in the instrument. Direct determination of trace elements in complex biological matrices leads to carbonaceous residue formation in graphite tubes which produce an intense background signal and affect analytical repeatability. To avoid such problem, serum samples were diluted (1+4) in HNO_3 and Triton X-100 solution as supported in previous studies [13-15, 18].

Optimization of heating program is one of the most important steps to provide a reliable and sensitive ETAAS method. In order to obtain a thermal stabilization of selenium in serum matrix and to get an integrated peak profile, pyrolysis, atomization temperatures and pyrolysis hold time were optimized. Selection of optimal values of heating program was conducted by varying one of these parameters while keeping the others fixed.

Two levels of drying step were performed in order to evaporate water solution. Thereafter, the influence of different pyrolysis temperatures on selenium was evaluated.

The absorbance signals of five-fold diluted serum with 60 ppb added selenium at different pyrolysis temperatures are summarized in Figure **1**.

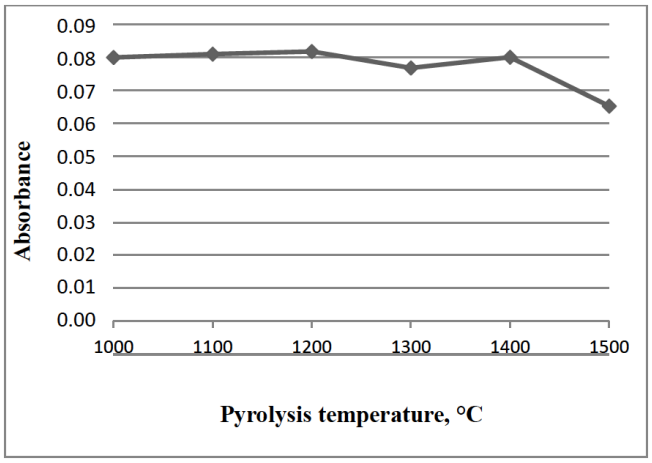

Figure 1: Pyrolysis temperature curve of selenium. 1.2 pg Se in 1: 4 diluted serum was used as test solution. Pd: 5 µg + $Mg(NO_3)_2$: 3 µg was used as chemical modifier.

Atomization temperature in the pyrolysis experiment: 1900°C.

Hold time pyrolysis: 11 seconds.

No significant losses of selenium was observed by increasing the pyrolysis temperature from 1000 to 1400 °C which suggest chemical modifier efficiency in increasing thermal stability of selenium. Therefore, a pyrolysis temperature of 1400 °C was considered as the optimal condition in this step of heating program.

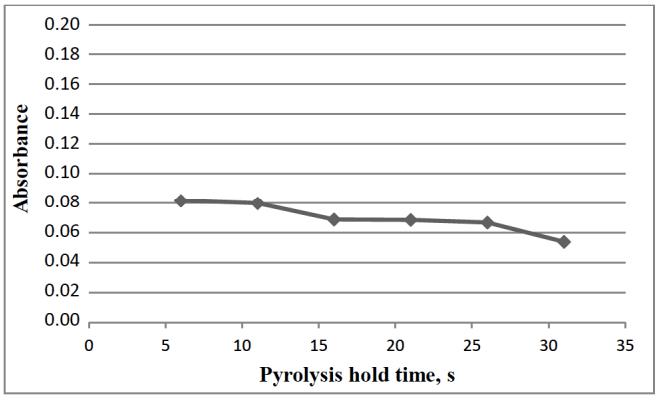

Figure 2: Pyrolysis hold time curve of selenium. 1.2 pg Se in 1: 4 diluted serum was used as test solution. Pd: 5 µg + $Mg(NO_3)_2$: 3 µg was used as chemical modifier.

Pyrolysis temperature and atomization experiment in the pyrolysis hold time experiment: 1400, 1900 °C respectively.

Since a slight decrease in the specific absorption signal was recorded for 11 seconds compared to lower pyrolysis time and a considerable decrease in the specific absorption signal was occurring for extended duration, this pyrolysis time was chosen for subsequent measurements (Figure **2**).

Figure **3** shows the results of heating program optimization according to atomization temperatures. This experiment indicates that the most practical pyrolysis temperature is 2000 °C.

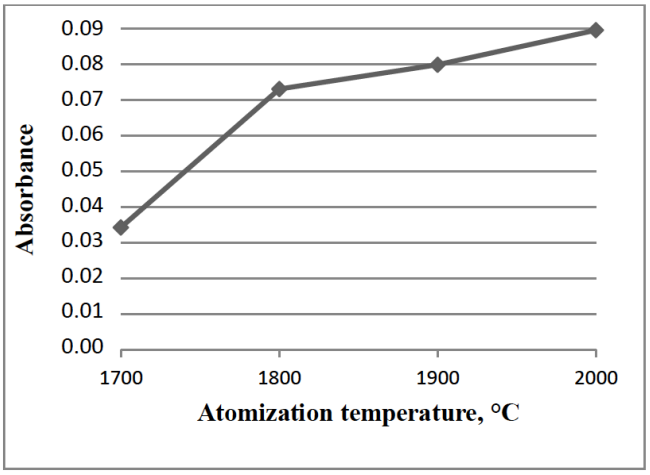

Figure 3: Atomization temperature curve of selenium. 1.2 pg Se in 1: 4 diluted serum was used as test solution. Pd: 5 µg + $Mg(NO_3)_2$: 3 µg was used as chemical modifier.

Pyrolysis temperature in the atomization experiment: 1400°C.

Hold time pyrolysis: 11 seconds.

3.2. Linearity

In the present study, the quantification of selenium was performed by means of the standard additions method. The standard concentrations were expressed

as the quantity added to undiluted serum (addition range: 50-300 ppb) with its corresponding signal. The highest standard should increase 1.5 to 3 fold matrix signal. Thereafter, the original amount of selenium in serum matrix was determined and adjusted calibration curve was deducted. The first order regression equation obtained was y = 0.001110417 x – 0.00073853 with correlation coefficient of 0.9997.

The linearity of the adjusted calibration curve was evaluated by the lack of fit test at 5% level of significance. According to F_{reg} value, the regression explains the observed variation (2359.831>Freg (5%)). The Fnl value indicate non-significant lack of fit of the calibration curve (0.103< $F_{lack\ of\ fit}$ (5%)).

Therefore, the regression equation establishes a linear relationship between concentrations and detector signals in the tested range. Thus, physiological and toxic levels of selenium can easily be determined by direct injections of diluted samples in graphite tubes.

3.3. Accuracy, Precision and Recovery

In this study, precision and accuracy were determined by intra- and inter-day validation using quality control samples at each point of the addition calibration curve.

The intra-day validation were performed by three runs with three replicate measurements of quality control samples on the same day. For inter-day validation, two runs with three replicate measurements on three successive days were performed.

Recoveries were calculated by comparing the found added concentrations of selenium in the quality control samples with nominal added concentrations. All recoveries were based on three replicate measurements.

Analyte concentrations in the quality control samples were calculated using the regression equation of the adjusted calibration curve.

Precision, accuracy and recovery expressed values of the analytical method were determined and the obtained results are summarized in Tables **2** and **3**.

All the coefficients of variation (CVs%) of intra-day and inter-day measurements were not greater than 5%. These results indicate a multilevel high precision of the present method regardless of the time factor.

All accuracy related results of intra-day and inter-day validation were not greater than 5% which also indicate a multilevel high accuracy of the present method regardless of time factor.

Table 2: Precision and Accuracy of the Method

Sample concentration (ppb)	Nominal added concentrations (ppb)	Precision (CV%)		Accuracy (relative bias%)	
		Intra-day assay	Inter-day assay	Intra-day assay	Inter-day assay
19.660	0	1.65	2.52	2.86	4.31
	10	4.93	2.50	1.93	1.30
	20	3.62	3.02	2.80	1.36
	30	4.02	2.40	1.21	0.88
	40	3.34	0.63	0.84	0.29
	50	3.44	3.23	1.39	0.34
	60	3.47	2.03	2.09	1.56

Table 3: Recovery Values of the Method

Sample concentration (ppb)	Nominal added concentrations (ppb)	Found concentration (ppb)	Recovery (%)
19.660	10	10.573	105.73
	20	18.888	94.44
	30	29.395	97.98
	40	40.502	101.25
	50	49.027	98.05
	60	58.333	97.22

Moreover, the intra-day precision in all calibration range (repeatability) was acceptable at 5% level of significance according to Cochran test (0.291<C (5%)).

The inter-day precision (reproducibility) in all calibration range were also acceptable at 5% level of significance according to Grubbs test.

The recovery values (94.44-105.73) in all calibration range indicate that the determination of selenium was not affected by the suggested matrix effects when the standard additions method was applied.

3.4. Sensitivity and Limits of Detection and Quantification

An acceptable characteristic mass of 92.550 pg was obtained using the adjusted calibration curve.

The limits of detection (LOD) and quantification (LOQ) were calculated from the adjusted calibration curve using the slope and the standard deviation of the intercept (3 s(b0)/b and 10 s(b0)/b values respectively). The equivalent values of LOD and LOQ in serum were determined by taking the dilution factor into account.

The LOD and LOQ values (4.010 and 13.375 ppb respectively) indicate that selenium concentrations below physiological range can be detected and determined.

3.5. Matrix Effect

Matrix effect was examinated by analyzing selenium in aqueous and serum matrices. The heating program used for selenium determination in 0.14% nitric acid and 0.2% Triton X-100 aqueous **s**olution was different from that in serum matrix. In addition, aqueous selenium standards gave a significantly different calibration slope from selenium in serum matrix and the aqueous selenium calibration was with higher detector signals compared to those of serum selenium calibration. Therefore, the calibration was established by standard additions method.

3.6. Application

Several reference values of serum selenium concentration were reported and reviewed according to different regions which suggest an environmental influence on selenium status, particularly, selenium content in local food [13, 19-23]. Reference values according to geographic localization can be classified in high, medium and low selenium status (50-80, 80-110 and 110-145 ppb respectively). Otherwise, bio-monitoring data of exposed workers are limited with a BAT value of 150 ppb according to DFG (sampling time not fixed) [24].

As an application of the present method, selenium was determined in serum samples taken from healthy individuals from Northern-Center of Algeria (10 males and 10 females) aged from 23 to 39 years. The obtained selenium concentrations ranged from 86.883 to 133.955 ppb with a mean value of 110.824±11.898 ppb. These values are in the same range with other previous studies applied in other countries which can be classified as high selenium status (Taiwan: 113.3±31.1 ppb, 110.9±21.5 ppb; Canada: 142.9±16.1 ppb; USA: 136.10±18.8 ppb) [13, 25-27].

4. CONCLUSIONS

A direct method for selenium determination in serum samples by ETAAS was developed. Pre-treatment procedures were reduced to a single dilution step, shortening therefore, total analysis time and lowering potential selenium losses and contaminations. In addition, dilution approach avoided the formation of carbonaceous residues in graphite tubes which may affect analytical repeatability.

To minimize the matrix effect and spectral interferences, three main requirements was achieved: the use of a longitudinal Zeeman background correction, the choice of an adequate modifier and the optimization of heating program.

Validation experiments revealed that the present method was sensitive, accurate and precise with low limits of detection and quantification which allow the determination of selenium deficiency cases. Moreover, the range of calibration curve enables selenium determination at toxic levels.

The obtained recoveries indicate the efficiency of the proposed procedure for selenium determination in serum samples in spite of suggested matrix effect.

Therefore, this analytical method can be easily employed for routine clinical purposes. It was successfully applied for selenium determination in serum samples collected from healthy individuals.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors wish to express their deep gratitude to other members of the National Center of Toxicology for their skillful cooperation.

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Received on 22-03-2017 Accepted on 28-12-2017 Published on 08-02-2018

DOI: https://doi.org/10.6000/1927-5951.2018.08.01.3