

## Research Article


**VALIDATION OF TWO SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF SELENIUM IN FOOD SUPPLEMENTS - WITH APPLIED MICROWAVE DIGESTION METHOD A COMPARATIVE EVALUATION OF PERFORMANCE CHARACTERISTICS**

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**ABSTRACT**

Microwave digestion method was used to preparing selenium samples from food supplements for selenium (Se) determination by spectrophotometric methods. This digestion method provides acid digestion of the samples in a closed vessel device using temperature control microwave heating for the selenium determination by spectrophotometric methods. Two simple, sensitive and specific spectrophotometric methods for the determination of selenium at trace levels were validated and compared on the basis of their performance characteristics. The spectrophotometry method using 3,3'-diaminobenzidine hydrochloride was based on the formation of stable yellow-colored piaszelenol complex by reaction of Se (IV) with 3, 3'-diaminobenzidine hydrochloride, which is then extracted in toluene and detected spectroscopically at 420 nm. The method using Thionin (TN) was based on the reaction of Se (IV) with potassium iodide in an acidic medium to liberate iodine. The liberated iodine bleaches the violet color of Thionin (TN), and which was measured at 600 nm. The limit of detection and quantitation for Thionin (TN) were 0.18 µg/ml and 0.56 µg/ml, respectively, and for 3,3'DABH were 0.008 µg/ml and 0.027, respectively. The optimum assay conditions were investigated and the average recovery from spiked samples was 99.7% -100.9%. Good values of precision were obtained, interday RSD% for Thionin (TN) was 1.41% and for 3,3'DABH was 1.62%. The validated methods were used for estimation trace amount of Se(IV) in food supplements.

**Keywords:** Selenium, Microwave digestion, Spectrophotometric, Thionin, 3,3'Diaminobenzidine, Food supplement, Method validation.

**INTRODUCTION**

Selenium (SE) is a micronutrient for human and animals. The nutritional importance of selenium is object of recent discussion. On one hand, selenium is essential to life, since it plays a protective role against the effects of heavy metals and its deficiency causes cardiomyopathy and osteoarthropathy.<sup>1</sup> On the other hand, selenium excess can induce selenium silicosis and cancer.<sup>2</sup> The range of concentration between deficiency and toxicity is very narrow and strongly depends on the chemical form under which the metal is present.<sup>3</sup> Also the bioavailability ("the micro nourishing amount that is available to being assimilated in a physiologically useful form")<sup>4</sup> depends on the speciation, so that it is not easy to individuate the correct dose. The Recommended Dietary Allowances (RDA) refers to total selenium. According to Dietary Reference Intake<sup>5</sup>, RDA is 55 µg/day for adults; according to the German and Austrian Nutrition Society and Swiss Nutrition Association, it ranges between 30 and 70 µg/day, while in Australia varies from 10–15 µg /day for infants to 70–85 µg /day for adults.<sup>6</sup> The tolerable upper intake level for adults is set at 400 µg/day.<sup>7</sup>

The major sources of selenium in the environment are volcanic eruption, insecticides, fertilizers, smelting ceramics, metallurgical operation, glass rubber accelerators, paints, dyes and electronic goods. Certain industrial and agricultural processes release selenium as a by-product, and there are cases where selenium from

such sources has caused environmental disasters.<sup>8,9</sup> Selenium is also reported to be present in cigarette paper, tobacco<sup>10</sup>, and various cosmetic samples<sup>11</sup>. Selenium enters into natural water through seepage from seleniferous oil and industrial waste. Water drained from such soil may cause severe environmental pollution and wild life toxicity.<sup>12</sup> The toxicity, availability and environmental mobility of selenium are very much dependent on its chemical forms.<sup>13</sup> In many environmental matrixes, e.g. natural water and soils, the predominant oxidation states of selenium are Se(IV) and Se(VI). Precise knowledge of the amounts of selenium and its compounds present in a system is therefore required for accurate assessment of the environmental and biological impact of selenium. This has resulted in an increasing need for analytical methods suitable for their determination at trace levels. Because of its significance, several analytical methods have been reported for the determination of selenium. These methods include neutron activation analysis (NAA)<sup>14</sup>, x-ray fluorescence<sup>15</sup>, chromatography<sup>16</sup>, hydride generation inductively coupled plasma atomic emission spectrometry (HG-ICP-AES)<sup>17</sup>, catalytic kinetic method<sup>18</sup>, atomic absorption spectrophotometry (AAS)<sup>19</sup>, high-performance liquid chromatography (HPLC)<sup>20</sup>, graphite furnace atomic absorption spectrophotometry (GF-AAS)<sup>21</sup>. Most of the previous methods used dedicated and/or expensive instruments that are not available in most quality-control laboratories. Despite the above-mentioned sensitive



techniques, spectroscopic determination of trace levels of selenium still represents an attractive technique for the determination of metal ions in food supplement because of its simplicity, low expense, and its more readily available, particularly in underdeveloped countries.

The objective of the present work was to provide a method for the determination of selenium in food supplements by using microwave digestion method (this method used for the first time in Syria to determine selenium in food supplements) for samples preparing before determination selenium by validated spectrophotometric methods. The validation parameters were investigated in accordance with approved guidelines<sup>22</sup> to assess overall suitability of the methods.

## MATERIALS AND METHODS

### Apparatus

- HITACHI UV-visible spectrophotometers (model U-2900, Japan) with matched 1 cm quartz cells.
- Microwave digestion (Anton Paar GmbH, Germany).

### Chemicals and Reagents

All chemicals were of analytical reagent grade and were used without further purification; the solvent were of spectroscopic grade. Double- distilled water was used for preparing reagent solutions and samples. Thionin (TN) solution (0.01%) was prepared by dissolving Thionin (Eastman Kodak, CI 52000; 10 mg) in water containing hydrochloric acid (2 mol L<sup>-1</sup>, 1 mL); the dye solution was stable for 30 days. 3,3'diaminobenzidine hydrochloride (DABH) (Sigma Chemical Co.) was prepared by dissolving 0.25g in 50 mL acetone and stored in amber-colored bottle; this solution should not be used after 8 hr of its preparation; it is always preferable to use it freshly prepared. All laboratory glassware was kept overnight in 10% nitric acid solution for decontamination. Before use, this glassware was rinsed with ultrapure water and dried in a dust free environment.

### Food Supplements Formulations

The following food supplements preparations were purchased from the local market and used in the present study: Supratech tablets (MBI Pharmaceutical Co., Aleppo, Syria) labeled to contain 55 mcg of elemental selenium per tablet [sample A]; Vita selen tablets (Ibn Al-Haytham Pharmaceutical Co., Aleppo, Syria) labeled to contain 100 mcg of elemental selenium per tablet [sample B].

### Preparation of standard solution

A stock solution of selenium (IV) (1000 µg/ml) was prepared by dissolving 0.219 g Na<sub>2</sub>SeO<sub>3</sub> (Sigma Chemical Co.) in 100 mL water. Then 1 ml of this solution was diluted to 100 ml with double-distilled water to get 10 µg/ml stock solution of selenium (IV).

### Construction of the calibration curve

#### Method using Thionin (TN)

Appropriate amounts of selenium stock solution (10 µg/ml) were diluted with double-distilled water, yields concentrations of 0.5 µg/ml, 1.5 µg/ml, 2.5 µg/ml, 3.5 µg/ml and 4.5 µg/ml, which were used for the construction of calibration curve.

#### Method using 3,3'DABH

Appropriate amounts of selenium stock solution (10 µg/ml) were diluted with double-distilled water (pH was adjusted to 1.5±0.3 using 0.5mol ml<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>), yields concentrations of 0.1µg/ml, 0.2µg/ml, 0.3µg/ml, 0.5µg/ml and 0.7µg/ml which were used for the construction of calibration curve.

### Preparation of sample solution

#### Sample Digestion

##### *Closed-vessel microwave oven total digestion procedure*

Twenty tablets of each formulation were weighed and finely powdered. Total digestion of the samples was achieved by mixing approximately 0.5g sample with 6 mL concentrated nitric acid (HNO<sub>3</sub> 65%) and 2 ml of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 30%) directly in the vessels used in the microwave oven. The vessels were closed, placed in the oven cavity, and submitted to a four-step heating program:

1. Ramp 20 min Power 700 W, Hold 15 min, Fan 1.
2. Ramp 0 min Power 300 W, Hold 15 min, Fan 1.
3. Ramp 0 min Power 0 W, Hold 20 min, Fan 2.
4. Standing for 2 min.

After cooling, which took approximately 15 min, the vessels were opened and their contents were quantitatively transferred to 100-mL volumetric flasks. The solutions were diluted to volume with double-distilled water and, when necessary, filtered to remove any remaining silica.

The concentration of the metal of interest was determined in the solution after suitable dilution to fit the linear working ranges.

### Procedure

#### Method using Thionin (TN)

Sample solution containing 0.5-4.5 µg selenium was transferred into a series of 10-mL calibrated flasks. Potassium iodide solution 2% (1 mL) then hydrochloric acid 2mol L<sup>-1</sup> (1 mL) were added and the mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine. TN 0.1% (0.5 mL) was then added and the reaction mixture was shaken for 2 min. The contents were diluted to volume with distilled water and mixed well. The absorbance of the resulting solution was measured at 600 nm against distilled water. A blank was prepared by replacing the analyte (selenium)



solution with distilled water. The absorbance corresponding to the bleached color, which in turn corresponds to the analyte (selenium) concentration, was obtained by subtracting the absorbance of the blank solution from that of the test solution. The amount of the selenium present in the volume taken was computed from the calibration graph.

#### Method using 3,3'DABH

Sample solution containing 0.1-0.7 µg selenium was transferred into a series of 10-mL calibrated flasks. 3,3'DABH (0.5 ml) was then added and the reaction mixture was subjected to heating (at 70°C) for 20 min. The mixture was cooled, and pH was adjusted to 8.0±1.0 using concentrated NH<sub>3</sub> solution. Colored complex was extracted with 10 ml of Toluene. The absorbance of the resulting solution was measured at 420 nm against distilled water. A blank was prepared by replacing the analyte (selenium) solution with distilled water. The amount of the selenium present in the volume taken was computed from the calibration graph.

#### Selection of wave length

In order to ascertain the wavelength of maximum absorption ( $\lambda_{max}$ ) of selenium, different standard solutions of selenium (2µg/ml Se – using Thionin) and (0.4µg/ml Se – using 3,3'DABH) were scanned using spectrophotometer within the wavelength region of 380 – 780 nm against a blank (blank: was prepared by replacing the analyte (selenium) solution with distilled water). The resulting spectra were shown in fig 1, 2 and the absorption curves showed characteristic absorption maxima for selenium at 600 nm using Thionin (TN) and 420 nm using 3,3'DABH.

The two spectrophotometric methods was validated according to ICH guidelines for linearity, precision, accuracy, LOD and LOQ.<sup>25</sup>

#### Linearity

Under the optimized reaction conditions, the calibration curves for selenium, the absorbance of selenium versus the concentration, with the target reagents used in this work were constructed by analyzing a series of standard solutions of selenium. The assay was performed according to the general procedure described in the

experimental section. The regression equations for the results were derived by using the least-squares method. The curves showed a linear response for the range 0.5-4.5 µg/ml selenium with Thionin (figure 3) and 0.1-0.7 µg/ml selenium with 3,3'DABH (figure 4). The correlation coefficient (r) of the resulting calibration curves were 0.9902 and 0.999 with Thionin and 3,3'DABH respectively. The equations of this curves were  $Y=0.091X-0.0054$  and  $Y=0.77X+0.012$  with Thionin and 3,3'DABH respectively. The equations of the curves applied to calculate the unknown selenium concentration in the food supplements form while x is the concentration of selenium and y is the absorbance (table 1).

#### LOD and LOQ

LOD (k = 3.3) and LOQ (k = 10) of the method were established according to ICH definitions. LOD and LOQ of method are reported in table 1. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations:

$$\text{LOD} = 3.3 \text{ S/M}, \quad \text{LOQ} = 10 \text{ S/M}$$

S = the standard deviation of the absorbance of the sample

M= is the slope of the calibrations curve.

**Table 1:** Validation Parameters

Parameters	Results			
	Thionin (TN)		3,3'DABH	
Absorption maxima (nm)	600 nm		420 nm	
Linearity range (µg/ml)	0.5-4.5 µg/ml		0.1-0.7 µg/ml	
Standard Regression equation	$Y=0.091X-0.0054$		$Y=0.77X+0.012$	
Correlation coefficient	0.9902		0.999	
LOD (µg/ml)	0.16		0.008	
LOQ (µg/ml)	0.49		0.027	
Precision	Intraday (RSD%)	Interday (RSD%)	Intraday (RSD%)	Interday (RSD%)
	0.278	1.41	1.5	1.62

**Table 2:** Recovery Study – Sample A

Level of Addition (%)	Formulation (µg/ml)	Addition of standard selenium (µg/ml)	Thionin (TN)		3,3'DABH	
			Recovery (%)	Recovery (%) ± S.D.	Recovery (%)	Recovery (%) ± S.D.
80	55	44	99.46	99.7±0.23	101.01	100.9±0.12
100	55	55	99.63		100.96	
120	55	66	99.92		100.79	

**Table 3:** Recovery Study – Sample B

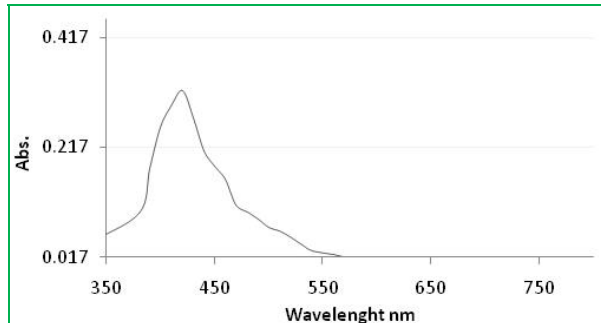
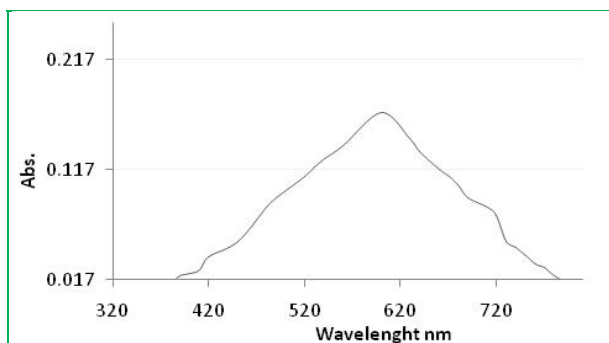
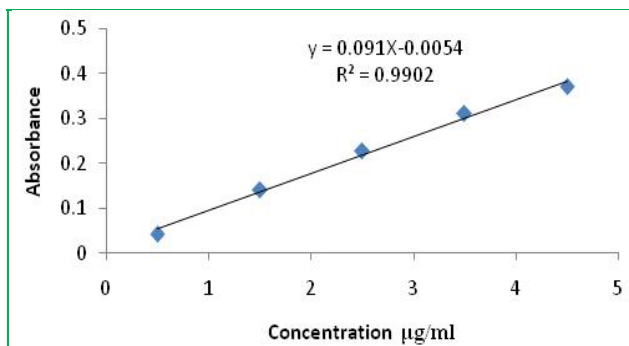
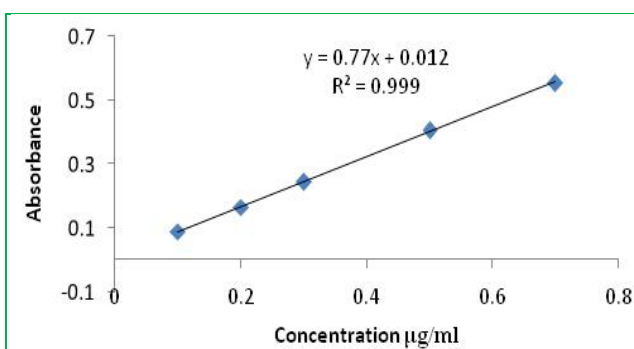
Level of Addition (%)	Formulation (µg/ml)	Addition of standard selenium (µg/ml)	Thionin (TN)		3,3'DABH	
			Recovery (%)	Recovery (%) ± S.D.	Recovery (%)	Recovery (%) ± S.D.
80	100	80	99.61	99.8±0.18	99.99	100.1±0.11
100	100	100	99.94		100.14	
120	100	120	99.89		100.21	



**Table 4:** Determinations of Selenium in Food Supplement (Tablets)

Sample	Label Claimed	Thionin (TN)		3,3'DABH	
		Amount found	Labeled Claim% *	Amount found	Labeled Claim% *
Sample A	55 µg/Tab	54.8±0.034	99.6	55.3±0.067	100.5
Sample B	100 µg/Tab	99.9±0.052	99.9	100.1 ±0.028	100.1

\* Average of three determinations

**Figure 1:** Maximum absorption ( $\lambda_{max}$ ) of selenium using Thionin (TN)**Figure 2:** Maximum absorption ( $\lambda_{max}$ ) of selenium using 3,3'DABH**Figure 3:** Standard curve of selenium using Thionin (TN)**Figure 4:** Standard curve of selenium using 3,3'DABH

## RESULTS AND DISCUSSION

The  $\lambda_{max}$  of Selenium was found to be 600 nm with Thionin (TN) and 420 nm with 3,3'DABH. From the optical characteristics (table 1) of the spectrophotometric methods, it was found that Selenium obeys linearity within the concentration range of 0.5 to 4.5 µg/ml and of 0.1 to 0.7 µg/ml with Thionin (TN) and 3,3'DABH, respectively, and coefficient correlation were found to be 0.9902 and 0.999 with Thionin (TN) and 3,3'DABH, respectively. The regression of the curves were  $Y=0.091X-0.0054$  and  $Y=0.77X+0.012$  with Thionin (TN) and 3,3'DABH, respectively.

The detection and quantitation limits as LOD ( $k=3.3$ ) and LOQ ( $k=10$ ) were calculated and these were found to be for Thionin (TN) 0.18 µg/ml and 0.56 µg/ml, respectively, and for 3,3'DABH were 0.008 µg/ml and 0.027, respectively.

The precision (measurements of intraday and interday) results showed (table 1) good reproducibility with percent relative standard deviation (RSD %) are below 2.0. This indicated that the method is highly precised. The percentage recovery value (table 2 and table 3), which was between 99.7%-100.9 %, indicates the accuracy of the methods and absence of interference of the excipients present in the formulations.

The spectrophotometric methods were applied for the assay of Selenium in tablets formulations of food supplements (in triplicate) and the results as tabulated in table 4. The results obtained were good agreement with the label claims.

## CONCLUSION

Summarizing the results, it can be stated that:

- Microwave digestion is a very useful method for digesting samples containing a low concentration of selenium. Due to the volatile nature of selenium, it is important to use the closed system. The microwave oven ensures safe and reliable digestions under appropriate conditions. Vessels stay sealed up to a pressure of 12-psig (830 kPa).
- The two validated procedures for selenium determination exhibit very good performance characteristics, especially in respect to accuracy and reliability, but each method does have a typical optimal application area as well as its specific advantages and limitations.

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