



## SPECIATION OF SELENIUM IN MEDICINALLY IMPORTANT PLANTS AND ITS BIOACCESSIBILITY

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### ABSTRACT

In present study highly sensitive and simple spectrophotometric method reported in the literature was modified and employed for speciation of selenium in medicinal plants. Total Selenium and bioaccessible selenium after *in-vitro* gastric and gastrointestinal method was determined using spectrophotometer and results were also verified with Inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique. The method involves reaction of selenium (IV) with 2, 3 di-aminonaphthalene to give the bright colored and strongly fluorescent 4, 5-benzopiazselenol compound. The optimum pH for this reaction was 2 and optimum temperature was 50°C. The molar absorptivity of the complex formed was  $29.866 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ . This method is applicable in the range of 25 - 2000 µg/l of selenium. Highest selenium content was found to be 32 µg/g in *Amoora rohitaka (Roxb)*. An *in-vitro* study indicates that bioaccessibility of selenium in *Sphaeranthus hirtus willed* was highest during gastro-intestinal digestion. This result shows that medicinal plants can be a good source of selenium bioaccessibility being high.

**KEYWORDS:** Selenium, speciation, bioaccessibility, ICP-AES, medicinal plants



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## INTRODUCTION

Medicinal plants are consumed worldwide for the treatment of several diseases and such plants are also an important source of raw material for pharmaceutical industries.<sup>1</sup> Despite numerous scientific works on mineral composition of medicinal and aromatic plants less data are available on speciation studies, especially on selenium. Selenium is one of the most vital trace elements, has a significant role in human diet acting as a preventative agent against some serious illnesses. It is a component of enzymes such as thioredoxin reductase and glutathione peroxidase.<sup>2</sup> The glutathione peroxidase is one of the antioxidant for body, which catalyzes some reactions and also inhibits the toxicity of few metals such as lead, mercury and cadmium.<sup>3</sup> Insufficient selenium intake causes an increased risk of thyroid, cancer, Keshan and Kashin-Beck disease.<sup>4,5</sup> Observational study indicated that death from cancer including lung, colorectal and prostate cancers is lower among the people with high content of selenium in the blood.<sup>6</sup> Recent studies on the relationship between the consumption of selenium and cancer prevention were mainly focused on the chemical form of selenium. Growing plants enriched with Se could be an effective way to reduce the dietary deficiencies and increase health benefits. The inorganic selenium species most frequently found are selenite [Se (IV)] and selenate [Se (VI)]. It is well established that selenium like all other trace metals is present in the environment in various chemical forms (speciation) whose toxicity may differ significantly.<sup>7</sup> The speciation of Se in plants involves selective and sensitive analytical techniques. Various instrumental techniques like HG-ICP-MS,<sup>8</sup> ICP-OES,<sup>9</sup> Neutron activation analysis (NAA)<sup>10</sup>, Atomic absorption spectroscopy (AAS)<sup>11</sup>, stripping voltammetry,<sup>12</sup> high pressure liquid chromatography (HPLC)<sup>13</sup>, gas chromatography (GC)<sup>14</sup> are available in the literature for the determination or speciation of selenium. However, spectrophotometry provides a simple and cost effective technique which can be used routinely for speciation. There are various reagents available in literature, like hydroxylamine hydrochloride<sup>15</sup>, 3-methyl-2-benzothiazolinone hydrazone hydrochloride<sup>16</sup>, azure B<sup>17</sup>, phenylhydrazine-*p*-sulphonic acid<sup>18</sup>, J-acid<sup>19</sup> for spectrophotometric determination of selenium (IV). The present work reports simple and sensitive spectrophotometric method using 2, 3 diaminonaphthalene for determination of selenium in different medicinal plants. Plant materials present a challenge for ICP-AES / Spectrophotometry analysis due to their complex composition. In order to break

down the matrix and incorporate the metal into solution the plant materials must be digested in acid solution. Bioaccessibility, previously termed as bioavailability, indicates the maximum fraction of a trace element or other substances in food that is theoretically released from the matrix in the gastrointestinal tract and thus available for intestinal absorption. Bioaccessibility of various elements is determined by either *in-vivo* administration to humans or *in-vitro* methods by simulating digestive system conditions in the laboratory.<sup>20</sup> In general, bioaccessibility is affected by the type and/or composition of food and also by the simulated gastrointestinal conditions (e.g., change in pH between the gastric and the intestinal track), which may affect the distribution of initial species. An adequate dietary intake of selenium would not necessarily mean that human body could absorb the whole amount ingested. In the past few years *in vitro* method to assess the bioavailability of minerals and trace elements have gained popularity because of its accuracy, speed of analysis and relatively low cost than *in vivo* experiments especially when there is an increasing interest in reducing the use of laboratory animals for testing.<sup>21</sup> India has a rich heritage of traditional medicines and the traditional health care system. It has several traditional medical systems such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. At present very limited data is available on speciation of selenium in Indian medicinal plants. Hence, the objective of the study is set to identify and quantify Se (IV), Se (VI) species along with total selenium and its bioaccessibility in medicinally important *Amoora rohitaka* (Roxb.), *Adiantum capillus – veneris* and *Sphaeranthus hirtus* Willd plants. Determination of Se was based on the reaction of Se (IV) with 2, 3-diamminonaphthalene in acidic medium and gives rise to greenish yellow piarselenol complex with characteristic absorption at 378 nm. Using above reaction Se speciation carried out along with bioaccessibility study, further all results were verified with ICP-AES technique.

## MATERIALS AND METHODS

### Apparatus

Total selenium concentration was determined by using spectrophotometer (UV-1800, Shimadzu) as well as ICP-AES technique. The ICP-AES instrument [M/s. SPECTRO, Germany] equipped with a cross flow nebulizer was used. Instrumental conditions for ICP-AES are given in Table 1.

**Table 1**  
**Instrumental conditions for ICP AES**

Nebulizer gas flow	0.8 L min <sup>-1</sup>
Auxiliary gas flow	1.0 L min <sup>-1</sup>
Coolant flow	12.0 L min <sup>-1</sup>
ICP RF power	1.6 KW
Rf frequency	27.12 MHz
Nebulizer	Cross flow type
Sample uptake rate	1.5 mL min <sup>-1</sup>
Plasma power	1400 W
Reading time	Auto

**Reagents**

All chemicals used were of analytical grade. Sodium selenite, 2, 3-diaminonaphthalene and hydroxylammonium chloride were procured from Merck, India. Pepsin, cyclohexane, potassium bromide and pancreatin were procured from SRL Chemicals, Mumbai and bile salts mixture was purchased from SD Fine Chemicals, Mumbai. Freshly prepared solutions of digestive enzymes (gastric and pancreatic) were used in the experiments. Deionized distilled water was used throughout the experiment.

**Solution of Se (IV)**

An aliquot of 1 mg/ml selenium solution was prepared by dissolving 2.190 g of sodium selenite in deionized distilled water containing 10 ml of conc. HCl and diluted to 1000 ml.

**2,3-diaminonaphthalene: [DAN reagent]**

2, 3-diaminonaphthalene solution was prepared by dissolving 100 mg of reagent in water with 500 mg of hydroxylammonium chloride by slow heating. The solution was then stored in an amber coloured bottle. Then in 50 ml of 2, 3-diaminonaphthalene solution, 25 mg of potassium bromide was added. The solution was filtered and used for further experiment as DAN reagent.

**Formation and extraction of piaszelenol complex**

In 10 ml of the sample solution / standard Se (IV) solution, 2 ml DAN reagent was added. Then the pH of solution was adjusted to 2 by drop wise addition of 1 M HCl solution. The solution was then kept at 50 °C in a boiling water bath for 5 minutes and allowed to cool at room temperature. The piaszelenol complex was extracted in 10 ml (5 ml X 2) of cyclohexane. The cyclohexane layer was dried over anhydrous sodium sulphate and the absorbance was measured at 378 nm.

**Collection and digestion of medicinal plant samples**

The medicinal plants used for investigations were purchased from local retailer. Authentication of all samples was done by botanist and voucher specimens for these plants have been deposited at the museum of the Agharkar Research Institute, Pune. The known amount of sample was crushed and ground to fine powder in mortar and pastel. For the determination of selenium content, 1 g of each sample was digested with 15 ml of a 5:1 mixture of nitric acid and perchloric acid. Then 10 ml water was added and the solution was filtered through Whatman No. 41 filter paper to remove any turbidity. To this filtrate 2–3 drops of HCl were added and the solution was diluted to 50 ml. All the

solutions were stored in tightly capped polythene bottles and were analyzed by ICP-AES technique.<sup>22</sup>

**Application of method to medicinal plant samples****Determination of Se (IV)**

For the determination of selenium (IV) from plant samples, the acid digested sample was allowed to react with DAN reagent, using 0.1 M EDTA solution as masking agent. All the optimized conditions were maintained and the piaszelenol complex was extracted in 10 ml (5 ml X 2) of cyclohexane. The cyclohexane layer was dried over anhydrous sodium sulphate and then absorbance was measured at 378 nm.

**Determination of Se (VI) and total Se**

4 M HCl was added to the sample extract and heated in a water bath at 60 °C in order to reduce Se(IV) and Se(VI) to Se. Concentration of selenium (VI) was then calculated by subtracting the concentration of selenium (IV) from total selenium.

**In vitro gastric digestion of samples**

The bioaccessibility of selenium was determined by *in vitro* gastric and gastrointestinal digestion method reported from our lab by Kulkarni et al.<sup>23</sup> For *in vitro* gastric digestion, 10 g plant sample was transferred to a beaker containing 100 mL of gastric juice solution (6% w/v pepsin 100 mL HCl of pH 1.75) and the mixture was shaken vigorously for 2 minutes. The flask was placed in a water bath at 37 °C on a magnetic stirrer cum heater. Temperature of water bath was adjusted such that the temperature of the reaction mixture placed in water bath was 37 °C. The reaction mixture was stirred continuously at a low speed for 3 hours. It was then centrifuged for 20 minutes at 2500 rpm and filtered through Whatmann No. 41 filter paper. The filtrate was analyzed for selenium using spectrophotometry techniques.

**In vitro gastro-intestinal digestion of samples**

For gastro-intestinal digestion solution obtained from gastric digestion was used and its pH was adjusted to neutral pH by drop wise addition of saturated solution of NH<sub>4</sub>HCO<sub>3</sub>. To this solution 75 ml of pancreatic solution (mixture of 2% w/v pancreatin and 0.2% w/v bile salts) was added. The mixture was again digested, as described above, at 37 °C for 4 hours. This solution was then cold centrifuged at 4 °C for 20 minutes at 2500 rpm. The supernatant was then filtered through Whatmann No. 41 filter paper and stored in an airtight container at 0–4 °C for further analysis. The % bioaccessibility (% B) of elements was calculated by using following formula:

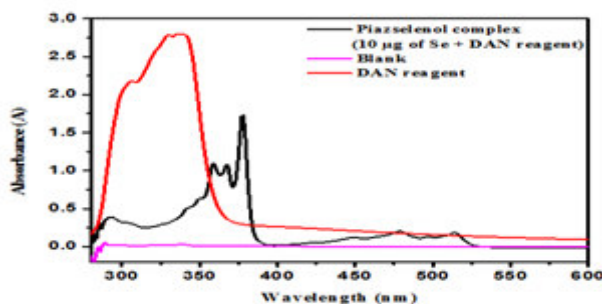
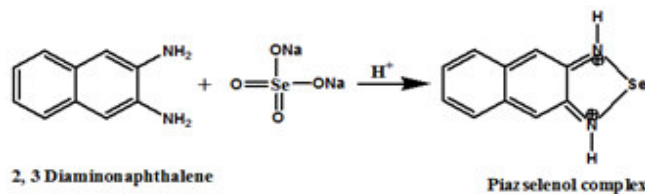
$$\% B = \left( \frac{[GD] \text{ or } [GID]}{T} \right) \times 100$$

where [GD] = Concentration of element in gastric digest, [GID] = Concentration of element in gastro-intestinal digest and [T] = Total elemental concentration in the sample.

**RESULTS AND DISCUSSION**

Parker and Harvey<sup>24</sup> first time found that the DAN to be a more suitable fluorimetric reagent for selenium

determination, but in present study we employed it for spectrophotometric determination of selenium. Selenium (IV) reacts with 2, 3 di-aminonaphthalene in acidic medium and gives rise to greenish yellow piaszelenol complex.



**Figure 1**  
**Absorption spectrum of DAN reagent, blank and piazselenol complex**

The complex was extracted in cyclohexane. The rate of the reaction was enhanced by small addition of bromide ions; it helps the quantitative conversion of other forms of selenium to Se (IV). Absorbance spectra for DAN reagent and piazselenol complex are shown in Figure 1. The major difference between these two spectra is, the absorption spectrum of complex extracted with cyclohexane shows an absorption maximum at 378 nm, which is the characteristic peak of compound of Se with DAN i.e piazselenol complex. Whereas the blank and DAN reagent has no absorbance at this wavelength.

The calibration curve was plotted using standard solution of Se (IV) at five different concentrations with regression value 0.997. The molar absorptivity of the complex formed was  $29.866 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ . This method is applicable in the range of 25 - 2000 µg/l of selenium. In order to confirm the accuracy of preparation of aqueous extract of MPs, the replicates samples of a MP ( $n = 3$ ) were spiked with standard solutions of Se. The accuracy and precision for the optimized method is shown in Table 2.

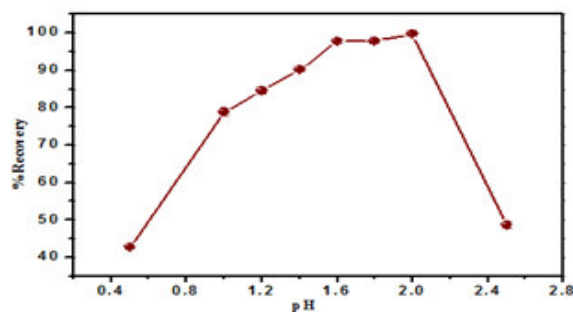
**Table 2**  
**The accuracy and precision of the method.**

Amount of se (IV) taken, µg/ml	Amount of se (IV) found, µg/ml	Relative error%	Relative standard deviation, %
4.00	4.06	+1.50	1.05
8.00	8.00	+0.00	0.00
10.00	10.03	+0.30	0.21
15.00	14.81	-1.26	0.90

#### **Effect of temperature and pH**

The reaction between Se (IV) and DAN reagent is markedly affected by pH. Se (IV) react with 2, 3 diaminonaphthalene in both acidic and neutral medium. According to Cambell<sup>25</sup> the optimum pH for reaction

between Se and DAN reagent is 1-2. In order to know the exact pH, the optimization between pH 0.5 – 2.5 was carried out and results are shown in Figure 2. It was observed that at pH 2 there was maximum complex formation and recovery of selenium was about 100 %.



**Figure 2**  
**Effect of pH on absorbance of piazselenol complex**

The effect of temperature on colour development reaction at laboratory condition was also studied. It was observed that at room temperature it takes long time for

colour development. Heating accelerates the time for reaching maximum absorbance but it also increases the rate of air oxidation of DAN. To avoid this, after reaching

pH 2 the samples were kept in boiling water bath for 5 min. The optimum temperature for the colour development was found to be 50 °C. Hence, further analysis of all the samples was carried out at pH 2 and temperature 50 °C.

#### Interference study

The effect of foreign ions on determination of selenium was studied by choosing 19 ions. For this purpose, small amount (5 ml) of standard solution (10 µg/ml) of Se (IV) was used in the presence of different amounts of foreign ions and the above described method was

used to determine selenium. The tolerance level for some selected ions is shown in Table 3. As table 3 demonstrates, large number of ions used has no considerable effect on determination of selenium except  $\text{Cu}^{2+}$ . From the experiment it reveals that 100 ppm of  $\text{Cu}^{2+}$  ions present in samples has no effect on selenium determination but above 100 ppm it interferes. The interfering effect of  $\text{Cu}^{2+}$  was removed by addition of small amount of masking agent (0.1 M EDTA solution). The masking agent helps to hold the most interfering ions in solution.

**Table 3**  
**Influence of some ions on the recovery of se (IV)**

Ion used	Added as	Tolerance level (ppm)	% Recovery <sup>a</sup>
$\text{Mg}^{++}$	Magnesium sulphate	2000	96.96 ± 1.45
$\text{Na}^+$	Sodium chloride	10,000	96.03 ± 4.00
$\text{Li}^+$	Lithium chloride	1000	98.12 ± 1.57
$\text{Al}^{3+}$	Aluminum sulphate	5000	99.32 ± 1.75
$\text{Ni}^{++}$	Nickel sulphate	2000	99.04 ± 0.90
$\text{Ba}^{++}$	Barium chloride	4000	99.08 ± 1.72
$\text{Co}^{++}$	Cobalt chloride	5000	97.27 ± 2.16
$\text{K}^+$	Potassium iodide	20,000	96.92 ± 1.59
$\text{Mn}^{++}$	Manganese sulphate	8000	94.98 ± 2.26
$\text{Ca}^{++}$	Calcium nitrate	50,000	98.23 ± 1.27
$\text{Pb}^{++}$	Lead nitrate	5000	97.03 ± 1.91
$\text{Ag}^+$	Silver nitrate	800	96.99 ± 1.82
$\text{Fe}^{3+}$	Ferric chloride	1500	97.23 ± 1.16
$\text{Cu}^{++}$	Copper sulphate	100	96.18 ± 1.12
$\text{Cr}^{++}$	Chromium sulphate	15,000	98.54 ± 1.40
$\text{Cd}^{++}$	Cadmium sulphate	20,000	96.69 ± 2.80
$\text{Cs}^+$	Cesium iodide	8000	98.27 ± 1.65
$\text{Zn}^{++}$	Zinc sulphate	20,000	97.34 ± 1.90
$\text{Sr}^{++}$	Strontium carbonate	7000	97.23 ± 1.57

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

#### Application to samples

Selenium can be present as elemental selenium ( $\text{Se}^0$ ), selenide ( $\text{Se}^{2-}$ ), selenite ( $\text{Se}^{4+}$ ), and selenate ( $\text{Se}^{6+}$ ). The  $\text{Se}^{4+}$  and  $\text{Se}^{6+}$  forms are both commonly found in natural, but out of these two species,  $\text{Se}^{4+}$  is more highly toxic. All these forms cannot be simultaneously

evaluated by direct application of certain analytical techniques. Hence we set our goal to evaluation of Se (IV) and Se (VI). The above described method was applied to medicinal plants for quantitative estimation of Se. Selected medicinal plants under study and their medicinal uses are listed in Table 4.

**Table 4**  
**Medicinal plants analyzed in present study**

Plant species	Family	Part used	Voucher No.	Medicinal use
<i>Amoora rohitaka</i> (Roxb.)	Meliaceae	Bark	S/B 101	Liver disorders, tumor, intestinal worms, skin diseases, diabetes, eye diseases, jaundice, resistance in breast cancer, colon cancer
<i>Adiantum capillus – veneris</i>	Adiantaceae	Leaves	WP 81	Urinary disorders, rheumatism, heartburn, gallstones, bronchitis, cough, and other respiratory problems.
<i>Sphaeranthus hirtus</i> Willd.	Asteraceae	Flowers	I/F 028	Used in tuberculosis, indigestion, bronchitis, spleen diseases, anaemia, pain in uterus and vagina, piles, asthma, leucoderma, hemicranias

Analysis of Se (IV), Se (VI) and total Se from medicinal plants by using spectrophotometer and ICP-AES technique are given in table 5. The ICP-AES is a rapid method and has very low detection limits, hence used to validate spectroscopy results. An examination of Table 5 shows that, all analyzed samples contain trace amount of Se.<sup>26</sup> The measured concentrations of total Se ranged from 16 µg/g to 32 µg/g. In all the analyzed plant samples it is observed that amount of selenium in selenite form is higher than selenate. Among the medicinal plants under study *Amoora rohitaka* (Roxb.) contains highest concentration of selenium (32.98 µg/g) and samples follows the order *Amoora rohitaka* (Roxb.) > *Adiantum capillus – veneris* > *Sphaeranthus hirtus*

Willd. In soil, Se most often occurs in soluble form such as selenate but our findings on medicinal plants shows that, maximum Se was present in selenite form rather than selenates. As reported in the literature addition of garlic and broccoli enriched with selenium in the diet may reduce the tumor incidence 60–90%.<sup>27</sup> Hence, selenium derived from medicinal plants used in traditional medicine can be used not only to prevent infection and other disorders, but also to treat them. It is therefore suggested that Se supplementation through reinforcement of endogenous antioxidative systems may be beneficial as an adjuvant therapy for some human pathologies.<sup>28</sup>

**Table 5**  
**Analysis of Se (IV), Se (VI) and total Se from medicinal plant samples ( $\mu\text{g/g}$ )<sup>a</sup>**

Samples	Se (IV)	Se (VI)	Total Se	
			By spectrophotometry	Total Se by ICP-AES
<i>Amoora Rohitaka</i>	16.61 $\pm$ 1.17	16.52 $\pm$ 2.09	32.98 $\pm$ 1.88	32.51
<i>Adiantum capillus veneris</i>	13.62 $\pm$ 1.32	08.59 $\pm$ 2.51	22.22 $\pm$ 1.42	20.45
<i>Sphaeranthus hirtus willd</i>	08.88 $\pm$ 0.44	07.53 $\pm$ 0.76	16.42 $\pm$ 1.09	15.75

<sup>a</sup> = Mean  $\pm$  standard deviation (N=3)

Bioaccessibility depends on several processes such as digestion, absorption, transport, utilization and elimination. Bioaccessible concentrations and percent bioaccessibility of the selenium determined from samples by *in-vitro* gastrointestinal digestion are given in Table 6. In all the cases, concentration of selenium in the gastric and gastro-intestinal digest is depended upon the total concentration of selenium in the original sample. For the medicinal plants under study, the concentration of Se in gastric digestion was found between the range 4.27 to 7.01  $\mu\text{g/g}$  and that for gastro-

intestinal digestion between 8.93 to 12.13  $\mu\text{g/g}$ . The % bioaccessibility values were observed in the range of 42.20 %, 39.43 % and 48.08 % for *Amoora rohitaka* (Roxb.), *Adiantum capillus – veneris* and *Sphaeranthus hirtus* Willd respectively in gastric digest. And that of further increase up to 73.02 %, 75.42 % and 100.96 % in gastro-intestinal digest. According to Oedrero et al<sup>29</sup> radish enriched with Se(IV) or Se(VI) shows bioaccessibility 70% in gastric digest and increased to 90 and 100% in gastro-intestinal digest.

**Table 6**  
**The bioaccessible concentration ( $\mu\text{g/g}$ ) and % bioaccessibility of Se from medicinal plant sample by *in vitro* method**

Samples	Bioaccessible concentration $\mu\text{g/g}$	
	Gastric digest	Gastro-intestinal digest
	<i>Amoora Rohitaka</i>	07.01 $\pm$ 0.66 (42.20)
<i>Adiantum capillus veneris</i>	05.37 $\pm$ 0.48 (39.43)	10.27 $\pm$ 1.21 (75.42)
<i>Sphaeranthus hirtus willd</i>	04.27 $\pm$ 0.59 (48.08)	08.96 $\pm$ 1.38 (100.96)

Values in parenthesis indicates percentage bioaccessibility

The bioaccessibility of selenium during gastro-intestinal digestion was higher than gastric digestion indicating higher absorption of minerals in gastro-intestinal tract at neutral pH. The gastric digestion procedure mainly breaks proteins into lower molecular weight peptides through the action of proteinases and peptidases. The intestinal digestion procedure through the use of pancreatin cleaves carbohydrates into monosaccharide and proteins into peptides. Finally, bile salts facilitate digestion of fats due to their emulsion forming properties.<sup>30</sup> A number of recent clinical studies carried out with selenium-enriched yeast show no evidence of toxicity following selenium intakes up to 343  $\mu\text{g}$  / day.

## CONCLUSION

In conclusion, spectrophotometric determination of selenium with DAN reagent was found to be highly selective. This method can be effectively applied to plant

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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