Determination of Selenium in Diet by Zeeman Effect Graphite Furnace Atomic Absorption Spectrometry for Calculation of Daily Dietary Intakes

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The selenium content of Turkish diets was determined by Zeeman effect graphite furnace atomic absorption spectrometry (GFAAS). Samples, collected from two different socioeconomic groups by duplicate portion technique, were digested with concentrated nitric acid in acid bombs, and hydrogen peroxide was added for complete oxidation. The digested solutions were then analyzed by GFAAS with the nickel chemical modifier addition. The detection limit for Se(VI) was 10.5 ng/mL for aqueous standard solutions. Average selenium concentrations of 0.069 ± 0.030 and 0.113 ± 0.036 mg/kg were obtained for the rural population group LALAHAN (n = 6) and for the urban population group METU (n = 10) samples, respectively. Daily dietary intakes of selenium were found as 23 ± 13 μg/day for LALAHAN subjects and 57 ± 31 μg/day for the urban population group (METU). The selenium intake for the first group is lower than the range of safe and adequate intake value, 50–200 μg/day, while that of the second group is just within the range.

The importance of trace elements in human nutrition has been well recognized for several decades with the demonstration of various deficiency syndromes. Selenium is one of the essential trace elements; however, some selenium compounds such as hydrogen selenide are extremely toxic and resemble those of arsenic in their physiological reactions.

Dietary selenium intake largely depends on the origin of the consumed foodstuffs. Meat and seafood tend to be rich in selenium, while the selenium content of vegetables depends mainly on the selenium concentration of the soil in which the plants were grown (Kajfosz and Szymczyk, 1984). The average safe and adequate daily dietary intake for selenium was given as 50–200 μg in 1986 by the National Research Council (1989).

Selenium is a constituent of an enzyme, glutathione peroxidase, that reduces lipid peroxides and hydrogen peroxide to alcohols and water, respectively. Its antioxidant activity is due mainly to its reduction of H_2O_2, which otherwise is reduced by Fe^{2+} to yield hydroxyl radicals, the main initiators of lipid peroxidation. It has been shown that there is a relation between the selenium content of diets and cardiovascular, cerebrovascular, coronary, ischemic, and hypertensive heart diseases. In addition, selenium may provide protection against some forms of cancer (Tölg, 1984; Ringdal et al., 1984).

The most commonly employed methods for the determination of selenium are instrumental neutron activation analysis (INAA) (Iyengar, 1985; Mumcu et al., 1988), proton-induced X-ray emission spectrometry (PIXE) (Kajfosz and Szymczyk, 1984), hydride generation atomic absorption spectrometry (HGAAS) (Julshann et al., 1982; Welz et al., 1984a–c; Brown et al., 1982; and Clinton, 1977), and graphite furnace atomic absorption spectrometry (GFAAS) (Ringdal et al., 1984; Julshann et al., 1982; Brown et al., 1982; Dillon et al., 1982); it is not an easy task to determine selenium if it is at trace levels, and many methods are still not fully adequate.

HGAAS and GFAAS are widely applied to the determination of selenium for concentrations down to 0.05 μg/g. The detection limit for GFAAS can be reduced by using a stabilized temperature platform furnace (STPF) and by means of Zeeman effect background correction (Carnrick et al., 1983; Voellkopf and Grobenski, 1984). HGAAS is suitable when the volume of the sample is unlimited. GFAAS has better reproducibility, is less time-consuming, requires small volume, is sensitive to nanogram levels and, therefore, is suitable for food analyses. However, since selenium is among the most volatile elements and is lost at temperatures above 300 °C, addition of a chemical modifier is needed.

In Turkey, studies on the determination of selenium in diets are not very extensive and few data have been published. Only Mumcu et al. (1988) and El-Hallaq (1988) have determined selenium in diet by INAA and RNAA, respectively.

In this study, analysis of selenium in Turkish diet by GFAAS was tried. Zeeman background correction was used to avoid the possible spectral interferences due to the presence of iron. Nickel was added as a chemical modifier.

EXPERIMENTAL PROCEDURES

Apparatus. A Hitachi Model 180-80 polarized Zeeman atomic absorption spectrometer equipped with graphite furnace system and an autosampler was used. A selenium hollow cathode super lamp manufactured by Photron was operated at 17.5 mA with a boost current of 40.0 mA. A spectral slit width of 1.3 nm was selected to isolate the 196.0-nm line. Peak area absorbance was calculated and displayed by a Hitachi 180-0205 data processing unit. Cup-type graphite cuvettes (Hitachi P/N 180-7402) suitable for samples containing organic substances were used. Argon served as the carrier gas at a flow rate of 250 mL/min, and it was interrupted during the atomization step. The sample volume injected for each reading was adjusted to 20 μL. Optimized furnace conditions are given in Table I.

Reagents and Standard Solutions. All solutions were prepared with analytical reagent grade chemicals. The Se(VI)

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Table I. Optimum Furnace Conditions for Se(VI)

stage	temp, °C	time, s
drying	70–100	20
drying	100–120	40
drying	120	40
pyrolysis	120–1000	60
pyrolysis	1000	40
atomizing	2400	7
cleaning	3000	3

stock solution was prepared from sodium selenate (Ventron Alfa Products). Five percent (w/v) nickel, prepared from $Ni(NO_3)_2 \cdot 6H_2O$ (Merck) was used as a matrix modifier. The standard solution of selenium was prepared daily in nitric acid, 5% (v/v) with 3000 mg/L Ni solution.

Preparation of Diet Samples. The duplicate-portion technique was used for the sample collection. Daily diet samples were collected from families of members of the Chemistry Department of the Middle East Technical University (METU) and villagers near Ankara (LALAHAN). Before samples were collected, a questionnaire was prepared for each subject, explaining the purpose of this investigation, giving the details of duplicate-portion technique, and collecting personal data. According to the criteria for selection of subjects, we have appointed one member from each of the LALAHAN and METU families to collect the samples.

We gave each individual three 3-L polyethylene containers precleaned with 0.2 N HNO_3. Samples were collected for 3 consecutive days, from the first morning to the third midnight for each subject. During the meal time, they were duplicating the amount of food equivalent to that consumed by them, putting the food on a plate, which was subsequently transferred to the polyethylene container with the spoon or fork, exactly in the eating style used by the subject. The container was stored in the refrigerator when not in use during this 3-day period to prevent odors and decomposition. Everything the subjects had consumed during this period was collected, including all of the meals and beverages. The uneaten parts like skins and seeds of fruits, bones, or oily residues were not collected. Details of sample collection and preparations are given by Mumcu et al. (1988) and Mumcu and Aras (1988).

Sample Treatment. A pressure decomposition technique was used to dissolve the samples. Approximately 0.4 g of sample and 4 mL of concentrated nitric acid were placed in a Teflon container. The system was heated to 120 °C in an oven and kept at this temperature overnight. Afterward, the volume was decreased to about 1 mL by heating at the same temperature. A few drops of H_2O_2 was added for complete oxidation, and the samples were finally diluted to 5 mL. Standard addition technique was used for the analysis of the samples with GFAAS. Nickel concentration was kept constant at 3000 mg/L for each sample.

RESULTS AND DISCUSSION

Optimization of Furnace Conditions. Most of the problems with the determination of selenium in GFAAS are related to losses prior to atomization; therefore, drying and pyrolysis stages must be carefully programmed. For the drying stage, several temperatures and times were tried, and the drying program given in Table I was found suitable for the diet samples to be analyzed. Selenium is typically found as Se(II) in biological materials and oxidized to Se(VI) state when digested with nitric acid and hydrogen peroxide. In our studies the conversion of Se(VI) to Se(IV) oxidation state was not complete; thus, the digested samples were analyzed directly without any reduction. As it has been stated by Welz et al. (1984a–c), different valency states of Se show different behavior; throughout this study Se(VI) standards were used for calibration. To find a suitable furnace program, behaviors of Se(VI) at different conditions were investigated. For this purpose, a 0.050 mg/L Se(VI) standard solution was prepared in 0.5% nitric acid, and its behavior was investigated at different pyrolysis temperatures in the presence of 3000 mg/L nickel. For Se(VI), a maximum signal was observed at 1000 °C. An atomizing temperature of 2400 °C was found optimum, regardless of its valency state. The detection limit for Se(VI) is 10.5 ng/mL, and sensitivity is 2.2 ng at 0.0044 au.

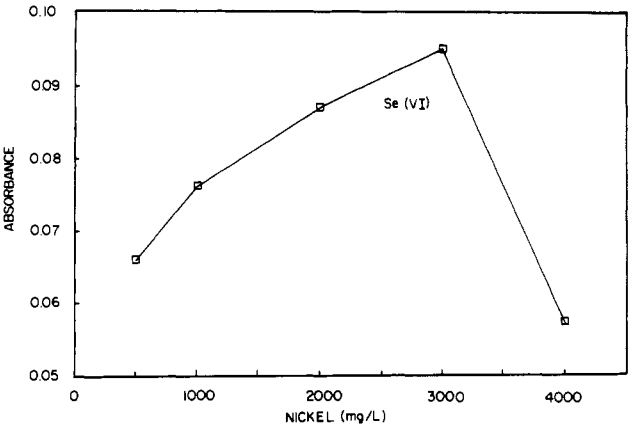

Figure 1. Effect of nickel concentration on 0.050 mg/L Se(VI) absorbance signal at pyrolysis and atomizing temperatures of 1000 and 2400 °C, respectively.

Effect of Nickel. Since selenium is a volatile element and lost above 300 °C, a chemical modifier was added to transform it into a thermally stable form. In the literature, several chemical modifiers such as nickel, copper, silver, molybdenum palladium, and different combinations of them have been proposed to stabilize selenium in the graphite furnace and thereby reduce losses and possible chemical interferences (Welz et al., 1984a–c; Saeed et al., 1979; Alfthan and Kumpulainen, 1982; Krynitsky, 1987; Cedergren et al., 1986). In this study nickel was used as a chemical modifier; the effect of different nickel concentrations on the response from 0.050 mg/L Se(VI) was investigated, and 3000 mg/L nickel was found suitable to stabilize selenium at optimized furnace temperatures (Figure 1).

Effect of Acidity. The effect of varying nitric acid concentrations on absorbance signals of Se(VI) was investigated. In the presence of 0.5% (v/v) nitric acid, there was an enhancement of the absorbance signal, and an increase in acid concentration from 1.0% to 5.0% resulted in a reduction of signal. Therefore, 0.5% (v/v) acid concentration was used in sample preparation.

Effect of Iron. In early studies, iron was used as a chemical modifier for selenium. Later, it was found that it was not a good chemical modifier since, at high concentrations, iron causes spectral interference on selenium at 196.0 nm when a continuum source is used for background correction (Manning, 1975). This spectral interference is always a problem, especially in the analysis of whole blood. The less sensitive 204.0-nm selenium wavelength or separation technique has been proposed to avoid the iron interference. In general, it is also asserted that these spectral interferences arising from the absorption of continuum radiation from the background corrector may disappear when Zeeman background correction is used (Welz et al., 1984a–c). The interference effect of iron on the absorbance signals of 0.050 mg/L Se(VI) was investigated, and it was observed that reduction of the selenium signal was less than 25% in the presence of iron even at a concentration of 1000 mg/L. However, it has been reported that the signal reduction is more than 50% with deuterium background correction (Çoruh, 1985). The average iron concentration of the diet samples was found to be 32 mg/kg dry weight by Mumcu et al. (1988) and Mumcu and Aras (1988). Therefore, there was no sig-

Table II. Comparison of Selenium Concentrations of LALAHAN Samples Analyzed According to Different Methods (mg/kg)

sample	this work	INAA[a]	RNAA[b]
LAL-1	0.057 ± 0.011	0.12 ± 0.03	0.062 ± 0.009
LAL-2	0.037 ± 0.003		0.038 ± 0.008
LAL-4	0.071 ± 0.017	0.08 ± 0.03	0.073 ± 0.011
LAL-9	0.047 ± 0.005	0.054 ± 0.010	0.051 ± 0.010
LAL-15	0.080 ± 0.021	0.064 ± 0.006	0.072 ± 0.013
LAL-20	0.121 ± 0.018	0.083 ± 0.041	0.110 ± 0.020

[a] Mumcu et al. (1988), instrumental neutron activation analysis. [b] El-Hallaq (1988), radiochemical neutron activation analysis.

Table III. Comparison of Selenium Concentrations of METU Samples Analyzed According to Different Methods (mg/kg)

sample	this work	INAA[a]	RNAA[b]
METU-1	0.066 ± 0.007	0.09 ± 0.01	
METU-2	0.095 ± 0.010	0.11 ± 0.01	
METU-4	0.091 ± 0.012	0.11 ± 0.03	
METU-6	0.126 ± 0.010	0.15 ± 0.04	0.13 ± 0.02
METU-7	0.097 ± 0.024	0.12 ± 0.04	
METU-8	0.175 ± 0.012	0.18 ± 0.04	0.19 ± 0.03
METU-9	0.153 ± 0.020	0.12 ± 0.03	0.16 ± 0.02
METU-10	0.097 ± 0.010	0.096 ± 0.029	0.084 ± 0.018

[a] Mumcu et al. (1988), instrumental neutron activation analysis. [b] El-Hallaq (1988), radiochemical neutron activation analysis.

nificant interference of iron on the selenium signal when studied with Zeeman background correction.

Sample Decomposition. Various decomposition procedures were tried to find the most suitable one for the determination of selenium in the diet samples. Among these procedures, nitric acid digestion followed by hydrogen peroxide addition gave the most successful results. Although the samples were destroyed completely in all other digestion procedures, the selenium signals were small and were not reproducible. In addition, they were not applicable to routine analysis, since a large volume of acids was used and reduction of this volume from 40–50 to about 6–7 mL was very time-consuming.

Daily Dietary Intakes of Selenium. IAEA-RM animal muscle (H4) was used to test the quality of the data. The selenium content of H4 was found to be 0.28 ± 0.06 μg/g, which is in good agreement with the certified value of 0.28 ± 0.05 μg/g.

Selenium concentrations of LALAHAN and METU diet samples were compared with those determined by INAA and RNAA (Tables II and III). Results are given as the mean value and the standard deviation of concentrations of five replicates. As seen, there is good agreement between the results of different analysis methods for both LALAHAN and METU samples.

From the concentrations of diet samples and the dry weights of total diets, the daily dietary selenium intakes were calculated for LALAHAN and METU groups and are listed in Table IV. The average daily selenium intake was found to be 23 ± 13 μg/day for the LALAHAN group and 57 ± 31 μg/day for the METU group. These results are different at 95% confidence level when Student's t-test is applied. When these values are compared with the recommended range of safe and adequate intake value of 50–200 μg/day, it is seen that the LALAHAN group has a lower intake than the recommended value and the METU group is just within the range. This means that the diet consumed by the lower income rural LALAHAN group is poor in selenium sources such as protein-rich foods. However, the diets consumed by the average income urban METU group were relatively rich in selenium sources of food compared to LALAHAN group. Daily dietary intakes

Table IV. Daily Dietary Selenium Intakes of LALAHAN and METU Subjects

sample	daily Se intake, μg	sample	daily Se intake, μg
LAL-1	14 ± 3	METU-1	25 ± 3
LAL-2	17 ± 1	METU-2	62 ± 6
LAL-4	27 ± 6	METU-4	25 ± 3
LAL-9	16 ± 2	METU-6	44 ± 3
LAL-15	15 ± 4	METU-7	41 ± 10
LAL-20	47 ± 7	METU-8	115 ± 12
		METU-9	87 ● 11
		METU-10	59 ± 6
av ± SD	23 ± 13		57 ± 31
range	14–47		25–115

Table V. Comparison of Daily Dietary Intakes of Selenium in Turkey with That in Other Countries (Kojfosz and Szymczyk, 1984)

country	daily Se intake, μg
Turkey	
LALAHAN	23 (this work)
METU	57 (this work)
United States	132
Canada	131
United Kingdom	60
Japan	88
Germany	30
Finland	30
New Zealand	18–26
Venezuela (Villa Bruzual)	300–400

of selenium for both LALAHAN and METU groups were also compared with those of other countries (Table V). The relatively low daily dietary selenium intake of our subject groups may be due to the low selenium content of the soil or the dietary habits. This question is under further investigation.

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