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Synthesis of Selenocystine Complex for Medicine Application

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Abstract: ***The selenocystine complex was biologically prepared by using local yeast; the prepared complex was characterized by using appropriate physical measurement i.e. FAAS, FTIR spectroscopy and melting point and analyzed by reversed phase HPLC and was compared with authentic standard. To treatment the deficiency level of selenium in hypertension and myocardial infarction patients, the complex was formulated at estate of drug and medical appliance in Sammara (in Iraq) as tablets. The present study involved the following groups: 1-100 healthy volunteers(55 men, 45women), 2-50 patients with myocardial infarction (men) and 3-50 patients with hypertension (men). The concentration of selenium was determined in the blood serum of healthy and patient's volunteers by using graphite furnace atomic absorption. The results showed that patients with hypertension and myocardial infarction had lower concentration of selenium in blood serum as compared with the healthy group. We supplied (20) patients with myocardial infarction and (20) patients with hypertension with (200mg) selenocystine complex tablet for three months. The results showed the selenium concentration in blood serum of hypertension patients were elevated from (42-53ng/ml) to (71-63ng/ml) and for myocardial infarction patients, the concentration were elevated from (35-45ng/ml) to (72-66ng/ml).***

Keywords: Synthesis, Complex, Medicine, Yeast

1. Introduction

Amino acids are critical to life and have many functions in metabolism. One particularly important function is the building blocks of protein. Amino acids are also important in many other biological molecules, such as forming parts of coenzymes as in s-adenosylmethionine, or as precursors of the biosynthesis of molecules such as hem [1].

There are some problems in the field of chemical synthesis of selenoamino acid compounds such as; environmental impact, energy loss and safety that need to be urgently tackled. Since biosynthesis are natural and high efficient, here is an example of how to solve some of these chemical problems through biological synthesis of selenoamino acid [2]. In 1984, Patrik and his collegagues [3] described the synthesis of selenocystine and selenohomocystine with o-acetylhomoserine sulfyldrylase, partially purified from baker's yeast. The enzyme was found to catalyze the synthesis of L-selenocystine and L-selenohomocystine from Na_2Se_2 with the corresponding acetyl derivatives of serine and homoserine. These researchers previously reported synthetic procedures giving optically active Se-substituted selenocysteines and selenohomocysteine using bacterial methionine ɣ-layase and tryptophan synthase. These enzymes could not be used in the direct synthesis of selenocystine and selenohomocystine [3].

Elements from various complexes with amino acids, these complexes have many biological effects and contributed in several metabolisms in human body [4]. Novel derivatives of selenoamino complexes that are effective dietary sources of supplemental selenium in humans and livestock are prepared and they have improved the biological properties and these complexes possess enhanced bioavailability sources of selenium [5].

In the recent years, attention has been paid to the role of essential trace elements like Se, Cu, Fe, Zn, Mg, Cr and V in ischemic heart disease (IHD), myocardial infarction (MI) and Angina pectoris (AP). Epidemiological studies have been shown that high intakes of mineral such as Se, Mg, Zn, V and Cr lead to reduced risk to cardiovascular disease. These exert a strong influence on individual risk factor for cardiovascular disease, such as disorders of blood fats, pressure and blood clotting [6].

2. Materials and Method

1- **Chemicals:**
All the chemicals used in this work were of highest purity available and were purchased from commercial sources (BDH and Sigma) without further purification.

2- **Physical measurement of the prepared ligand (Cystine) and its complex**
FTIR: The infrared spectra of the cystine and selenocystine complex were recorded using **Shimadzu FTIR (8000), with potassium bromide (KBr) disc in the wave number in (4000-400) cm^{-1} range and with (CsI) disc in the wave number (400-250) cm^{-1}range.**
HPLC: The high performed liquid chromatography used in this work was **Shimadzu (Japan, Koyot, Co)**. It was equipped with a LC-18 guard column (4.0 mm X 3.0 mm i.d, 40µm particles size) and followed by a supelcosil LC-18 column (150 X 4.6 mm i.d, 5µm size from supelco, Bellefronite. PA). Samples were injected using a Rheodyn 7125 U.S.A. sample injector with 750µL loop.

Identification and quantization was accomplished using UV-Visible detector model SPD-6A set at 230 nm. Control of HPLC system was through LC-6A controller. The integration of the chromatographic peaks and gradient HPLC system was run at 1 ml/min and a mobile phase composition of acetonitrile :

ammonium phosphate buffer 0.01 M (90:10 v/v). The sample injection volume was 750µL.

A Shimadzu model AA670 atomic absorption spectrophotometer was carried out for determination of selenium equipped with Shimadzu (GF-4B) Graphite furnace atomizer for volatization and atomization in place of flame. A Shimadzu Koyota . Japan, single-element hallow cathode lamp (H.C.L.) were used as spectral sources. Inert gas (Argon) was used as the purge gas with flow rate 1.5 L/min. Melting point apparatus of Gallen Kamp M.K.B-60 was used to measure the melting point of complex.

3. Preparation method of Selenocystine Complex

3.1 Preparation of Culture Media

The (10gm) yeast was grown on locally sterile liquid media containing (20gm) glucose, (20gm) peptone. The media was strengthened with 0.3% potassium phosphate (KH_2PO_4), 1% ammonium sulfate and inoculation locally culture media (and foreign for the purpose of comparison), with incubation at 30^0C for 24 hours in the shaker incubator at speed of 150 cycles/minute.

3.2 Addition of Selenium Salt

The solution of sodium selenite was added to the culture media in different concentrations (25, 50, 100 and 200µg/ml) to activate the growth process known as exponential phase. The above various doses were added to culture media during one hour for each dose and incubated at 30^0C for 24 hours. The process was shacked using shaker incubator at speed of 150 cycle/min for six hours before incubation. The above various doses were added to culture media during one hour for each dose and incubated at 30^0C for 24 hours. The process was shaked using shaker incubator at speed of 150 cycle/min.for six hours before incubation. The final product was a selenocystine complex of red color. It was filtered to remove the insoluble material accompanied the reaction product, and then washed with deionized water several times (3-4 times). Finally, it was washed with acetone and drying at 80^0C for three hours. In the present study, it noted that higher amounts (more than 200µg/ml) of sodium selenite in the culture medium have a strong inhibitory effect on the growth of this yeast. The chemical formula of complex: $C_6H_{12}N_2O_4Se_2$

M.P.=227^0C, Yield=42%, (Found 46.80%Se, Cal. 47.29%Se), Found 21.81%C, Cal. 21.55%C, 3.72%H, Cal. 3.59%H, Found 8. 81% N, 8.55%N, Cal. The formed product is obtained using the local design reaction vessel which consisted of the following units:

1. Pyrex container 20 L includes growth media and aqueous salt.
2. Incubator fixed at 30^0C.
3. Vacuum pump.

4. Sample Introduction

An injection method was used for this purpose. In brief, a small volume (200-250µL) of diluted serum was injected with micropipette ependoff into a small Teflon funnel connected to nebulizer by a short piece of capillary tube. This method overcomes some problems encountered in the classical continuous aspiration methods due to ability of high volume of serum sample that would be clogging the nebulizer and burner system because of a high salt content and viscosity effect on analytical accuracy.

5. Results and Discussion

1- Synthesis and properties of the complex

The complex in this study is important because it is useful as organic supplemental forms for human body; it is less toxic and higher absorption by gastrointestinal tract than supplemental forms.

2- Biosynthesis of selenocystine complex by yeast

The chemical analogy between organic compounds of sulfur and those of selenium suggest a relationship between the natural derivatives. Painter and Frank [7] indicated that the selenium derivatives can be seleno amino acids analogous to the naturally occurring sulfur amino acids. The hydrolysis of protein containing selenium showed that the selenium is found in the fractions containing cystine.

Accordingly, yeast has been chosen in our study to synthesis of selenocystine complex within cell, because it efficiently accumulates inorganic selenium salt (selenite) and subsequently metabolizes it to less toxic organic compounds seleno amino acids depending on the culture conditions [8]. This is due to replacement of the sulfur atom of the amino acid (cystine) by selenium.

It has been found that introduction of water- soluble selenium salts as a component of the culture medium for yest produced by conventional batch processing result in a substantial amount of selenium being absorbed by the yeast and different concentration of selenite dissolved in water to the growth culture, taking into consideration the situation to avoid discouraging the growth of yeast. It is important to note that the growth condition of yeast including pH and carbon source (which regulate selenite uptake by the yeast).

3- Infrared Spectroscopic study

The FTIR spectra data of ligand and prepared complex were listed in table (1). For the ligand, the band was observed at (3027) cm^{-1} can be assigned to stretching vibration for the (NH_2) group [9] while the bending vibration appeared at (1583) cm^{-1} for the NH_2 group. The carbonyl group (C=O) stretching vibration appeared at (1623) cm^{-1} [9] and a strong band was observed at 1292 cm^{-1} belong to (C-O) bond. The hydroxyl group (O-H) vibration appeared at (3523) cm^{-1} [10] and a strong band was observed at (673) cm-1 and (538) cm-1 assigned to the (C-S) bond and (S-S) bond respectively. For the prepared no appreciable change in the absorption of NH_2 group of the complex, the band was observed at (3037) cm^{-1} and (1585) cm^{-1} belong to stretching and bending

vibration respectively, which exclude the possibility of nitrogen atom of amine group to participate in coordination with metal ion. No change in the absorption of carboxylic group of the complex, which exclude the possibility of oxygen atom of the carboxylic group to participate in coordination with metal ion. A new band appearance at (392) cm^{-1} attributed to (C-Se) bond. This confirms the replacement of the sulfur atom of amino acid by selenium [3]

Table 1: The most diagnostic FTIR bands of ligand and its complex in (cm^{-1})

Group	Ligand (Cystine)	Selenocystine complex
(N-H) stretching	3027	3037
(N-H) bending	1583	1585
(C=O)	1623	1623
(C-O)	1292	1296
(C-S)	673	-
(S-S)	538	-
(C-Se)	-	392
(O-H)	3523	3525
(C-H)	2860	2922

6. Analysis of Complex by HPLC

The prepared complex was analyzed by reversed phase HPLC concentration was measured quantitatively by comparison and the the peak area of the authentic standard with that of the prepared selenocystine under the same separation conditions the percentage of synthesis was (42.6%) as show in the chromatogram see figure (1).

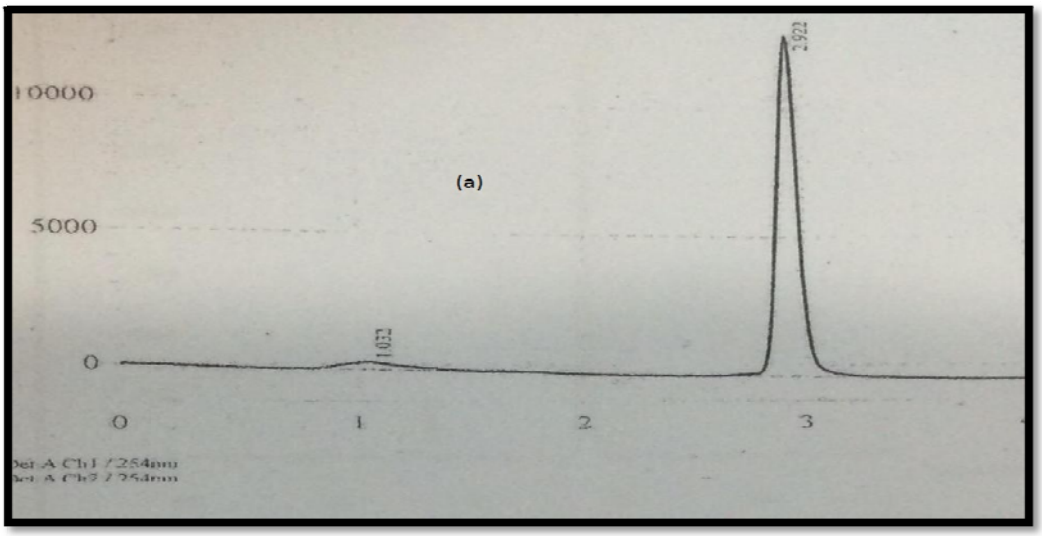

Figure 1 (a): HPLC of Cystin

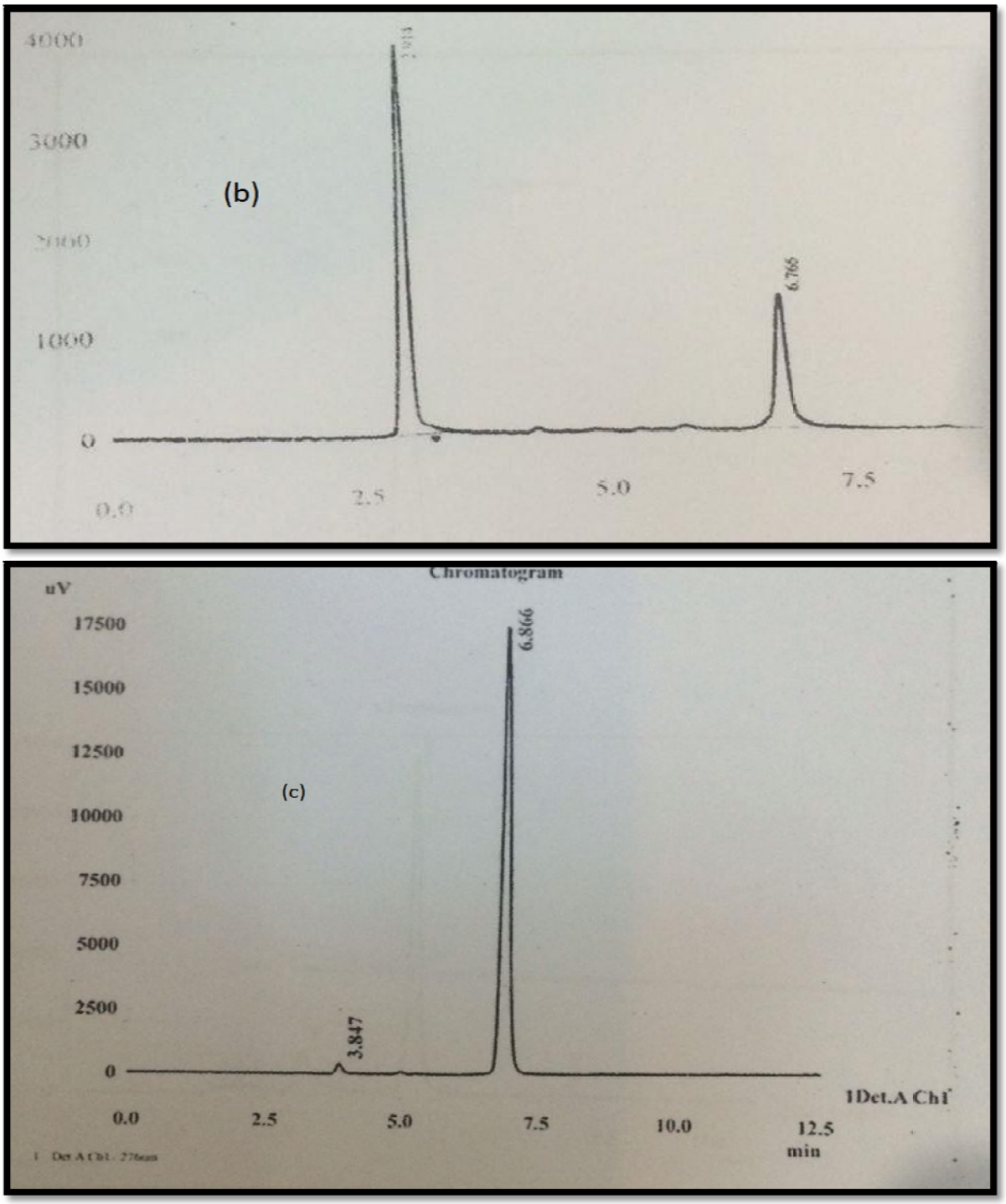

Figure 1 (b): HPLC Of the prepared Selenocystine complex (c) HPLC of the standard Selenocystine complex

The retention time of selenocystine was (6.8 min.) compound with the retention time of cystine the same separation conditions the result observe the retention time of cystine was (2.9 min.). The difference in retention time between cystine and selenocystine complex was a good indication for formation of selenocystine complex.

7. Determination of Selenium in Human Serum

Serum sample is diluted two fold with deionized water. One set of calibration standard solution are prepared in the (20-150 ng/ml) program for A Shimadzu flameless atomic absorption spectrophotometer of selenium was showed as below:

Wave length (nm) 196.0
Spectral band width (nm) 0.3
H. C. L. current (mA) 12
Drying(ramp) temperature (^{0}C/sec.) 150/30
Ashing(step) temperature (^{0}C/sec.) 900/4
Atomization(step) temperature (^{0}C/sec.) 2000/3
Gas flow (L/min) 1.5
Signal mode peak height
Sample volume (µL) 20

Standard solutions of the selenium prepared ranging from 20-150 ng/ml Se. 20 µl of the standard solution was injected in the furnace, followed by 20 µl of the chemical modifier

solution. GFAAS is the most widely employed method for determining selenium.

A pyrolysis (decomposition) temperature of 900^{0}C can be used for selenium for a short time (4 sec.) which makes it relatively simple to remove the bulk of the matrix prior to atomization without any loss for analyst. The recommended atomization temperature is 2000^{0}C for (3 sec.), the conditions recommended here are suitable for direct determination of selenium in serum specimens. Peak height has been preferred for the absorbance of signal measurements because of its high sensitivity especially in very low concentrations of analyst (Ultra-trace elements). The volatility of selenium makes it subjected to loss from the graphite furnace if the ashing temperature is too high or the heating cycle is too long. The sensitivity was found to be 2.5 ng/ml for selenium per 1% absorption and correlation coefficient was r=0.9991.

8. Estimation of Selenium in Healthy Group

The selenium concentrations were measured in healthy volunteers in both sexes between 20-over 60 years. The results show a slight decrease in selenium with increase ages for both sexes as seen in tables (2-4) show the comparison between concentrations of selenium in both sexes at various ages between 20 over 60 years, the histogram fig (2) was illustrated the ranges of selenium concentration for both sexes.

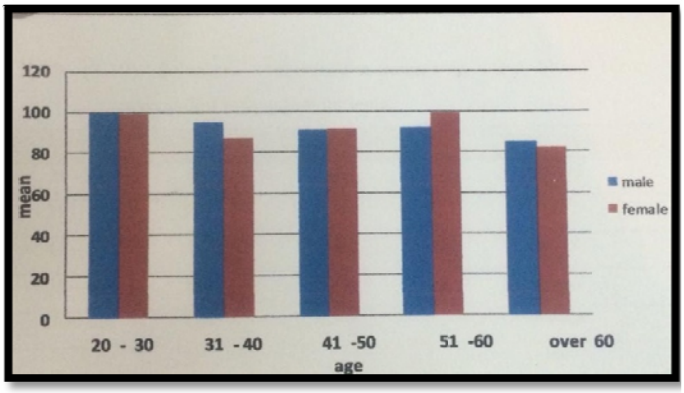

Figure 2: Comparison of selenium concentration in blood serum of both sexes adult healthy volunteers.

Table 2: Selenium concentration ranges in male blood serum of various healthy volunteers

Age	No.	Range ng/ml	Mean ± SD
20-30	8	54-106	100 ± 22
31-40	12	56-98	95 ± 31
41-50	15	53-94	91 ± 36
51-60	10	58-95	92 ± 34
Over 60	10	55-86	85 ± 27
	55		n.s

Table 3: Selenium concentration ranges in female blood serum of various healthy volunteers

Age	No.	Range ng/ml	Mean ± SD
20-30	8	59-150	99.01 ± 36
31-40	10	54-150	87.3 ± 27
41-50	7	53-160	91.5 ± 32
51-60	12	53-155	99.2 ± 31
Over 60	8	56-147	82.0 ± 20
	45		n.s

Table 4: Comparison of Selenium concentration in blood serum of both sexes adult healthy volunteers

Age	Male (mean) ng/ml	Female(mean) ng/ml	P-Value
20-30	100 ± 22	99.01 ± 36	n.s
31-40	95 ± 31	87.3 ± 27	n.s
41-50	91 ± 36	91.5 ± 32	n.s
51-60	92 ± 34	99.2 ± 31	n.s
Over 60	85 ± 27	82.0 ± 20	n.s

9. Estimation of selenium in patients groups

These are summarized in table (4)

Table 4: Selenium concentration ranges in male blood serum of various Hypertension patients

Age	No.	Range ng/ml	Mean ± SD
20-30	7	50-57	53 ± 3.5
31-40	11	47-52	50 ± 3.4
41-50	14	45-56	49 ± 3.0
51-60	9	42-54	45 ± 2.7
Over 60	9	41-49	42 ± 2.2
	50		n.s

Table 5: Selenium concentration ranges in male blood serum of myocardial infarction patients

Age	No.	Range ng/ml	Mean ± SD
20-30	9	40-51	45 ± 1.9
31-40	12	38-50	44 ± 2.3
41-50	8	37-48	38 ± 3.6
51-60	10	35-47	36 ± 3.1
Over 60	11	31-42	35 ± 2.8
	50		n.s

10. Selenocystine Supplementation

The concentration range of selenium in various ages of hypertension patients between 20- over 60 years were in the range of 42-53 ng/ml while for myocardial infarction patients were in the range of 35-45 ng/ml. Therefore, the deficiency of selenium was observed in most cardiovascular patients, since selenium activates. The immune system through the main enzyme glutathione peroxidase and selenoproteins, thus there is an urgent need for selenium supplements. The modern studies confirm that selenium complex was best selenium supplements in the viewed absorption and in renormalizing the level of selenium in human beings [11]. Therefore, selenocystine tablet 200 µg formulated in Estate for drug and msdical appliance, Sammara (IRAQ) was used for the two groups of patients. The first group involved hypertension patients. The selenium level elevated from 42-53 ng/ml to 63-71 ng/ml after supplying these patients with 200 µg selenocystine tablets for three months, whereas the second group involved myocardial infarction patients. The selenium level elevated from 35-45 ng/ml to 60-72 ng/ml after supplying the patients with selenocystine complex 200 µg tablets for three months, as noted in table (6) and (7).

Table 6: Selenium concentration ranges in male blood serum of various hypertension patients after supplying them with 200 µg selenocystine tablet for 3 months

Age	No.	Range ng/ml	Mean ± SD
20-30	4	58-82	71 ± 6.2
31-40	5	57-80	70 ± 5.4
41-50	7	52-77	65 ± 2.7
51-60	4	55-75	63 ± 4.2
	20		n.s

Table 7: Selenium concentration ranges in male blood serum of various myocardial infarction patients after supplying them with 200 µg selenocystine tablet for 3 months

Age	No.	Range ng/ml	Mean ± SD
20-30	5	56-78	72 ± 4.7
31-40	6	55-76	68 ± 5.6
41-50	4	54-71	71 ± 4.6
51-60	5	51-66	66 ± 4.21
	20		n.s

11. Selenium Recovery Study

The accuracy of the method has been investigated by using recovery experiments. Table (8) shows that the determination of selenium added to serum by GFAAS results in recoveries ranging from 95.94% to 97.47% taking into consideration the (RSD%) reported. The results presented are the average of three determinations. It has been concluded that the conditions recommended here are suitable for direct determination of selenium in human.

Table 8: Analytical recovery of selenium added to serum

Added ng/ml in final volume	Found ng/ml	Expected ng/ml	Recovery %	RSD %
0	98	-	-	5.2
50	142	148	95.94	4.2
100	194	198	97.47	3.4
120	211	218	96.7	6.1

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