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RESEARCH ARTICLE

DETERMINATION OF SELENIUM IN WHOLE BLOOD OF CARDIOVASCULAR DISEASE PATIENTS IN BASRA CITY BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY.

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Abstract

A developed hydride generation atomic absorption method for the determination of selenium using a Micro reaction vessel of a volume 10ml and 13mm in diameter for the analysis of small volumes of biological fluids such as whole blood and serum. The optimum conditions used for determination were(1.5 M HCl medium),N_2 Flow(2L/min) reducing agent (2%w/v $NaBH_4$).It has been found that the detection limitof the method(1.36ng/ml),sensitivity(0.045ng/ml),linear (0.0- 200 ng) with recovery (82.7-106)%for whole blood sample. The results reveals that low selenium contents of the whole blood of male patients (40,84 ng/ml) and 33.33ng/ml for female patients as compared with healthy whole blood (95.34ng/ml) For male and (82.0ng/ml)for female respectively.

Introduction:-

Selenium is an essential trace element and an important constituent of some endogenous enzyme glutathione peroxidase which represent an extremely effective protective System for insufficient [(1)]activity of enzyme can be the reason for metabolic disturbance of the body cells and serves as a radical scavenger. It has been found that peroxy radicals Play a decisive role in inflammatory, degenerative and chronic illnesses. Because of its antioxidant properties, it has long been hypothesized that selenium may prevent cardiovascular and other chronic diseases. There are an association between a lower antioxidant intake with greater incidence of heart diseases A additional lines of evidence suggest that oxidative stress from free radicals may promote heart diseases[(2)] In this research developed direct method for the determinations of Se in whole blood has been used, the method was found to be rapid accurate and sensitive.

Reagents:-

1000ppm selenium solution was prepared from selenium dioxide 1%W/V sodium borohydride solution was prepared by dissolving 1 g of sodium borohydride in 100 mL of the deionized water it was freshly prepared . 1.5M HCl solution was prepared by diluting 147.3mL of. concentrated (2M) HCl (1 M)HNO3 solution was prepared by taking 6.8 mL from the concentrated solution and diluted to 50 mL by deionized water (1:1)HCl and HNO3 Acid solution was prepared by taking one volume of each and diluted with one volume distilled water

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Instruments:-

The shimadzo atomic absorption spectrometry model AA 630–12 was used for the analysis and a home-made hydride generation kit also used as shown in figure(1)

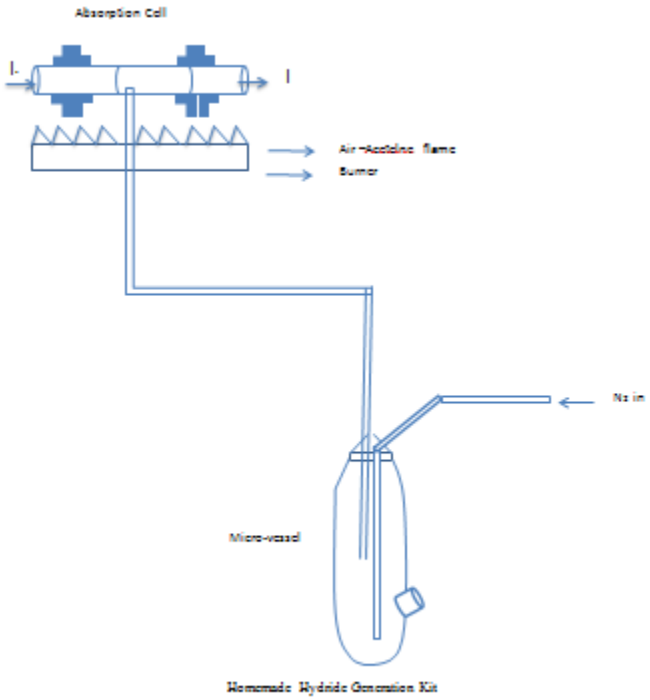

Figure 1:- Home made hydride generation kit

General procedure:-

40ng of selenium were placed in a reaction vessel by taking 0.2 mL of(200 ng per ml) selenium solution Then 0.2 mL of (2% W/V) sodium borohydride solution was added to the reaction vessel the content were mixed well by shaking and Selenium were liberated hydride was pushed by nitrogen gas with rate of 3L/min to the reaction cell and atomic absorption signal of selenium was measured using 196.1 nanometer wavelength The effect of other factors such as flow rate of nitrogen gas , volume and concentration of reductant different heating time of absorption cell different selenium concentration and different volume of selenium all these factors were optimized as shown in figure 1,2,3,4 and 5

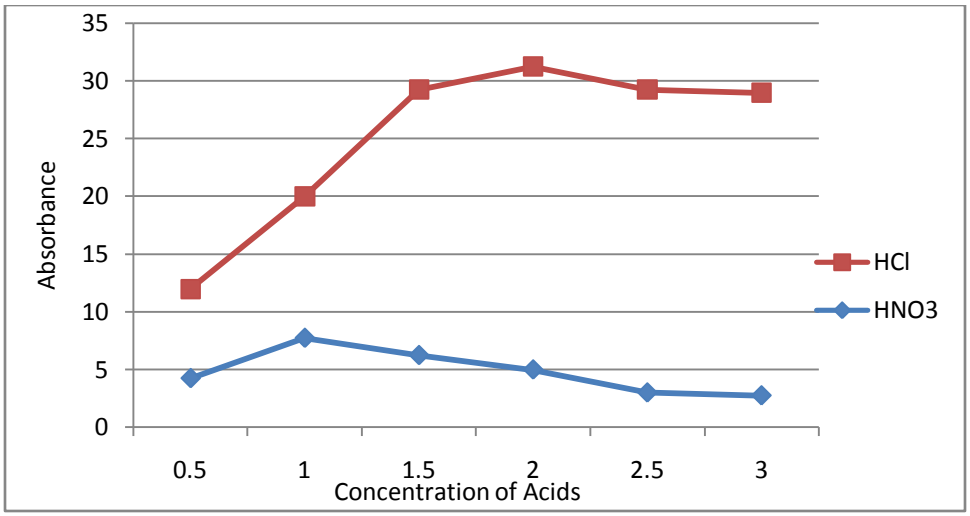

Figure 2:- Effect of acids on the absorbance of 40ng Se.

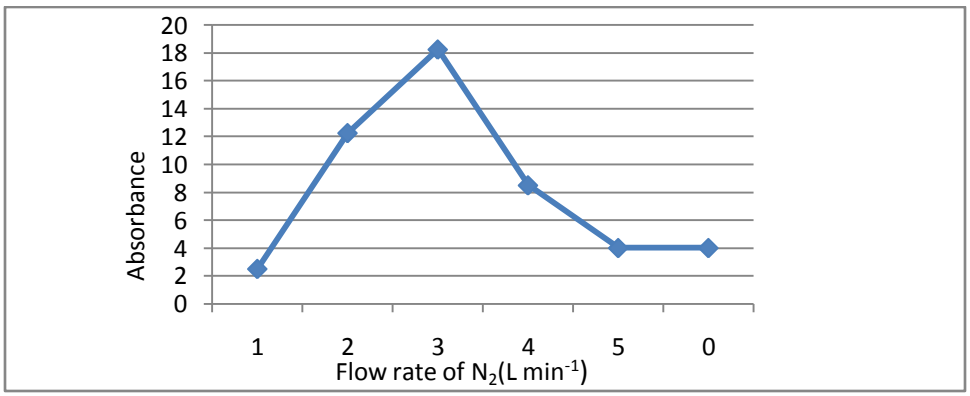

Figure3:- Effect of different flow rate of N_2 on the absorbance of 40ng Se

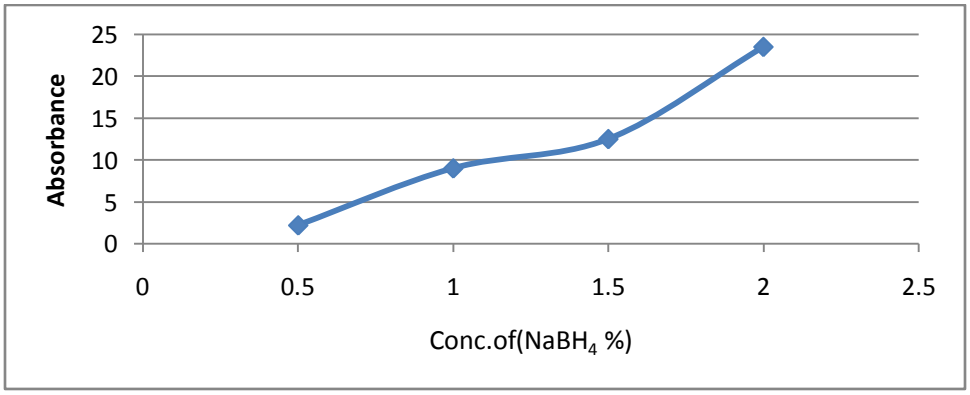

Figure 4:- Effect of different Conc of $NaBH_4$ on the absorbance of 40ng Se.

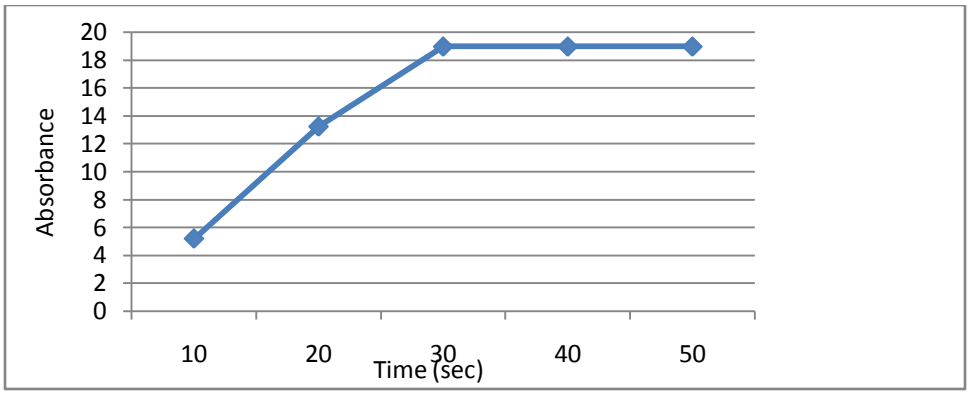

Figure 5:- Effect of heating time of absorption cell on the absorbance of 40ng Se

Sample collection:-

Blood samples were collected from 178 cardiovascular patients and healthy control person these samples were related to different life spans and different gender and the local of collection was basra hospital and some health centers trees the blood sample was kept in polyethylene vials containing potassium ethylene diamine tetra acetic acid as coagulant and stored in a refrigerator at -20°C till analysis

Sample digestion:-

0.5mL of blood sample 2 mL concentrated HNO_3 were added to it then solution was butted in a water path (100-110°C) till complete dissolution and the solution become clear(2-3hrs) this solution was diluted with 1.5M HCl to 5 mL Then heated in a water bath at(60 - 70°C) for (20 -30) minutes to convert all selenium 6 to Se four Then the sample were cooled then diluted to 5mL with 1.5M HCl.

Table 1:- Optimization conditions for the determination of Se using NaBH4 as reductant.

Optimum Conditions	Parameters
1.5M	HCl concentration
30Sec≥	Heating time
2%	Concentration time
0.2ml	Volume of $NaBH_4$
3L Min^{-1}	N2 Flow rate
0.2ml	Volume of standard solution

Construction of selenium calibration graphs:-

0.2mL of selenium solution was injected into a reaction vessel followed by adding 0.2 mL of 2%W/V sodium borohydride solution using micropipette .The content of the vessel was stirring well and the nitrogen gas with the flow rate of 3L/min was used for pushing the liberated selenium hydrides to the absorption cell and the atomic absorption signal of selenium was measured using 191.1nm.

Results and Discussion:-

Optimization conditions for selenium determination:-

The atomic absorption signal of 40 ng selenium was measured using different acid media the results revealed that the use of HCl as acidic medium for the hydride generation gives higher atomic absorption signal than HNO3 acidic media figure.

Effect of different nitrogen flow rates the atomic absorption signal of selenium was affected by the purity of nitrogen ,impure nitrogen with purity less than 99% gives a background absorption which cover Se signal and then none reproducible signal were obtained therefore high purity of nitrogen 99.999% should be used and different flow rate used to force SeH2 to the absorption cell as shown in fig2 which reveals that 3L/min. was the best rate.

Effect of sodium borohydride concentrations:-
Figure 3 shows the effect of different concentration of sodium borohydride on the AA signal.of selenium, it's clear that the concentration of (2% W/V) sodium borohydride are sufficient to reduce the selenium +4 ions to SeH_2 and give the higher signal of Se.

The effect of different time of absorption cell:-
using different time for the heating of absorption cell gives different absorption signal for Selenium as shown in figure 4 the low temperature of heating using less time gives less signals and as the time of hearing increases the signal of selenium was increases it reaches maximum at 30 seconds of heating time fig.4

Calibration graph:-
we can construct the calibration by using different volume of a certain standards (200 ng / ml) se solution by taking different volumes of a standard solution to construct their calibration as shown in figure 5

Optimization conditions:-
The optimize conditions for the determination of selenium by hydride generation was shown in table 1 and under these conditions we can construct calibration graphs with a range(0-200) ng/ml as shown in figure 6.

The analysis methods
Two methods direct and standard addition are used for the determination of selenium in whole blood which indicated in table 2 and figure 8 and 9 The obtained results shows that a good compatibility between method and standard addition method. The accuracy of direct method was found to be(92-109)%. and recovery percent of.(82.7-106)% the direct method.was reliable when compared our result with other studies(6,7)

Generally the results show a low selenium content of the whole blood of male patients (40.84 ng/ml)and (33… ng/ml)for female as compared with healthy whole blood (95.34 ng/ml)for male and (82.0 ng/ml)for female respectively

Table 2:- Determination of selenium by direct method as compared with standard addition.

Accuracy %	Conc. Of Se in whole Blood (ng/ml) by Addition	Conc. Of Se in whole Blood (ng/ml) by direct	Sample
109	22	24	A
92.30	65	60	B
105	80	84	C
97.36	76	74	D
96.77	124	120	E
100	18	18	G
100	52	52	H
100	88	88	I
100	52	52	J
97.36	76	74	K
100	88	88	L

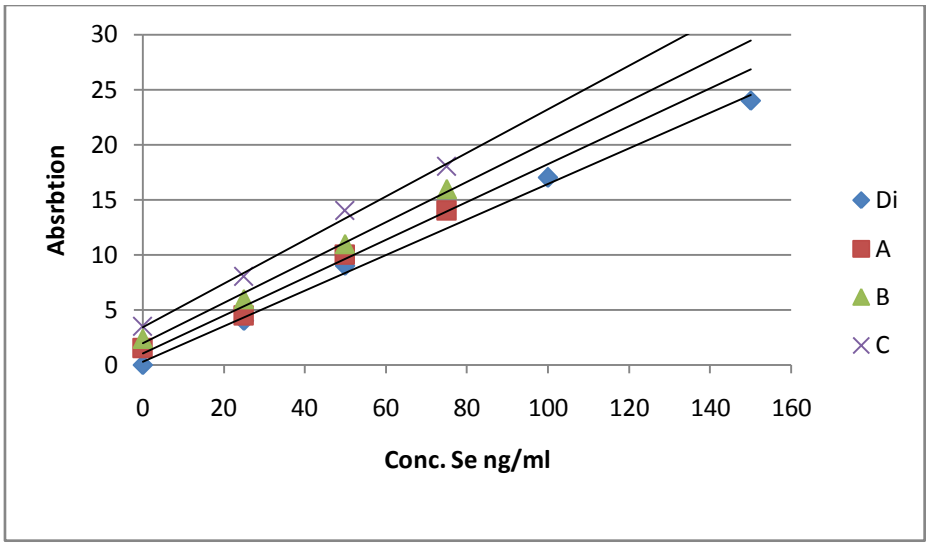

Figure 7:- Determination of Selenium in whole Blood by Direct Method and standard addition method

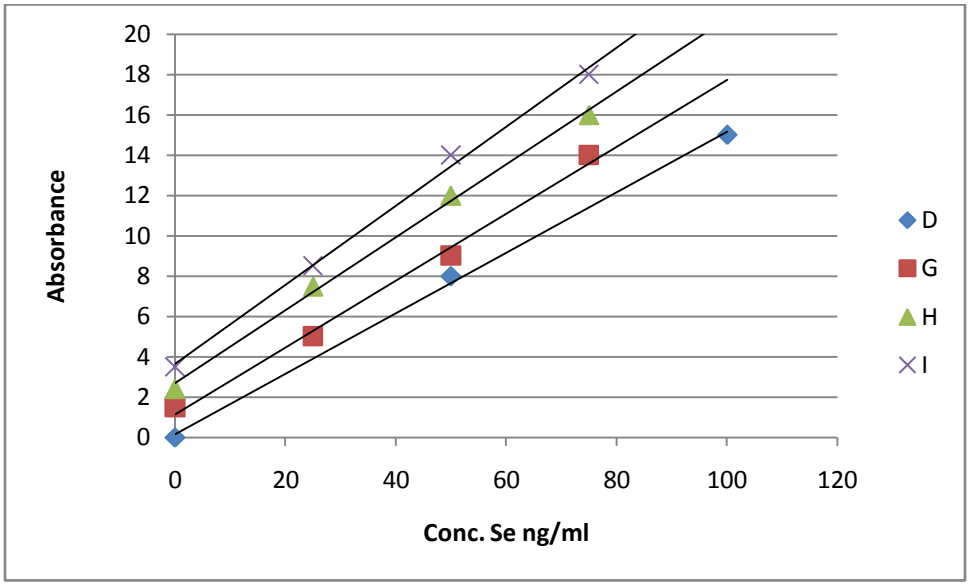

Figure 8:- Determination of Selenium in whole Blood by Direct Method and standard addition method

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