

DETERMINATION OF SELENIUM USING THE DITHIZONE REAGENT BY A NEW DESIGNED FOR MERGING ZONE AND SEQUENTIAL INJECTION TECHNIQUES

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ABSTRACT: A new and innovative design for a new Merging Zone Flow injection and Sequential injection systems has been developed to determine Selenium (IV) using Dithizone at the wavelength the greatest absorption 410nm. The detection limits and the linearity ranges were calculated where it was $0.029 \mu\text{g ml}^{-1}$, $0.039 \mu\text{g ml}^{-1}$ and $(0.05-10) \mu\text{g ml}^{-1}$, $(0.05-5) \mu\text{g ml}^{-1}$ and the sampling rate of 100, 90 samples per hour for FIA and SIA, respectively. The effect of various physical and chemical parameters for the two systems was studied. The designed systems were applied successfully for Se (IV) quantity determination in tap water and pharmaceutical formulations. The recovery average was which varies from 94.110 % to 103.676 % and 90.72 % to 102.24 % for FIA and SIA, respectively.

KEYWORDS: Selenium, Sequential injection, Determination, Dithizone.

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I. INTRODUCTION

Selenium is a cumulative non-metallic element present in high concentrations in food and water. It is an element necessary for normal cellular function, but it can have toxic effects at high doses. It has received great attention from researchers because of its dual role as an essential tracking element and toxic component [1,2]. Selenium speciation has gained much attention in recent years because Selenium compounds have been reported to have anti-cancer activity and prevent toxic effects of heavy metals [3,4].

The bioavailability and toxicity of Selenium depend on both its concentration and its chemical form like other trace minerals. In fact, all essential elements may be toxic to humans if ingested at high levels for a long time. Selenium is also known as an antioxidant in the diet, it is a component of antioxidant enzymes [5,6].

Selenium is existing as selenocysteine (Se-Cys) in at minimum 30 proteins, Proteins that contain Se-cys in their polypeptide chain are known as Selenoproteins and be in all lineages of life (archaea and bacteria), however, some organisms do not have the enable to synthesize selenoproteins [7,8]. Selenoproteins are central to human and animal health due mainly to their antioxidant activity [9]. They have chemopreventive, anti-inflammatory and antiviral properties and are associated with improved immune responses [10].

Selenium deficiency can lead to fetal cardiomyopathy Keshan disease and contributes (along with iodine deficiency) to Kashin-Beck disease. It is also necessary for the conversion of the thyroid hormone thyroxine (T4) into its more active counterpart triiodothyronine (T3) and as such this deficiency can cause symptoms of hypothyroidism, including extreme fatigue, goitre, mental slowing [11,12].

There are many different analytical methods for the determination of Selenium, such as Liquid-phase microextraction [13,14], UV-photochemical vapor generation with quartz furnace atomic absorption spectrometry [15], Cathodic stripping voltammetry [16], HPLC-ICP-MS [17], ICP-MS [18], Cloud Point Extraction [19].

The main purpose of this work was to design two new systems a Merging Zone Flow injection system FIA and the other a sequential injection SIA for determination the Selenium ion(IV) in water and pharmaceutical samples. This study gave low detection limits in addition to being sensitive, selective and inexpensive.

II. EXPERIMENTAL

2.1. Apparatus

The pH was measured using the Oakton2100 Series pH/mV/Ion/°C/°F Meter. Calculation of weight was performed using Ohaus PA214 Pioneer Analytical Balance. The two designed systems are shown in Fig. 1 and 2 consists of silicon tubing of 0.5 and 1mmi.d. was used for the loops of the lab-made valve, holding coil and reaction coil, They also contain a prismatic pump Ismatic was used to push the solutions, The flow cell used is 450 µL, The resulting peak was obtained by using a UV-Visible detector (OPTIMA SP300) and The recorder also records a signal Pen Siemens C1032 Hitter Ardeas 51.

2.2. Chemicals and reagent

All chemicals used were of analytical reagent grade. Selenium dioxide (purity: 99.9%) was obtained from Sigma chemicals company (Germany). Dithizone (purity: 98%) was obtained from Merck. hydrochloric acid (36.5–38%) was obtained from avantor performance materials. Pharmaceuticals were purchased from the market.

2.3. Standard and working solutions preparation

A stock solution (1000 mg L⁻¹) of SeO₂ was prepared by dissolving 1.4053 g of SeO₂ in one liter of distilled water, and additional dilution was performed to obtain working solutions.

Dithizone (Dz) stock solution 0.1% (w/v) was prepared by dissolving 0.1 g of Dithizone in 100 ml of 96% ethanol solution in the volumetric flask.

Sodium dodecyl sulfate solution SDS 0.01%(w/v) prepared by dissolved 0.01 g of salt in 100 ml water.

Triton X-100 solution 0.01%(v/v) prepared by dissolved 0.01 ml of Triton X-100in 100 ml water.

2.4. Recommended procedure

2.4.1. Merging Zone flow injection system FIA

The Selenium (IV) solution was loaded at a concentration of 10 µg mL⁻¹ and the reagent solution at a concentration of 0.01% was loaded on the two equal valve loops of size 157µL, the carrier current solution was pumped by the pump at a flow rate of 9.1ml min⁻¹.The absorbance of the mixture was measured and recorded at 410 nm.

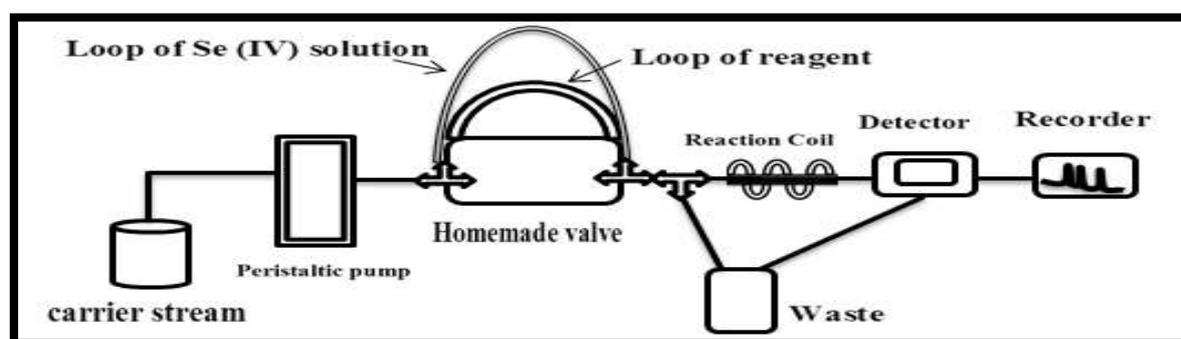


Fig. 1: Schematic Diagram of the Designed Merging Zone Flow System (FIA)

2.4.2. Sequential injection SIA

The Selenium ion solution was loaded at a concentration of $5 \mu\text{g mL}^{-1}$ and the reagent solution at a concentration of 0.01% was loaded on the two equal valve loops of size $157 \mu\text{L}$, then the solutions were pushed towards the holding coil using a medical syringe and the carrier current solution was pumped by the pump at a flow rate of 3.6 mL min^{-1} . The absorbance of the mixture was measured and recorded at 410 nm.

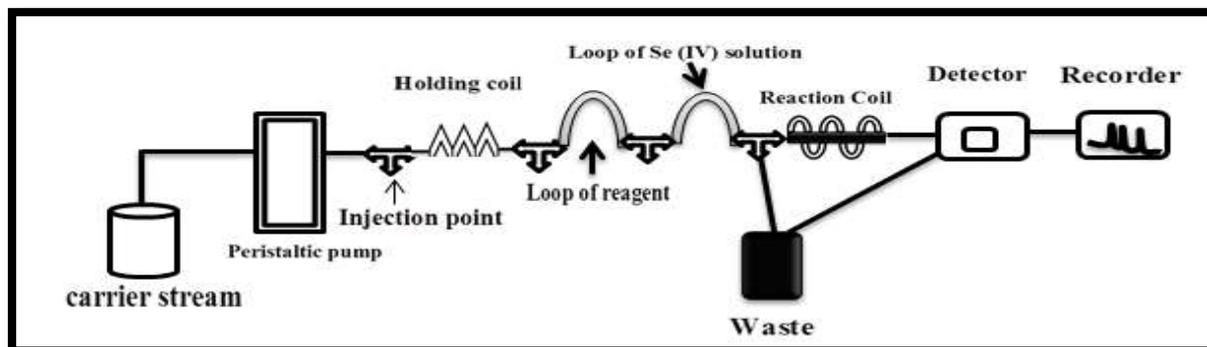


Fig. 2: Schematic diagram Of the Designed Sequential Injection Analysis SIA

2.5. Procedure for Pharmaceutical formulations

To obtain a $100 \mu\text{g mL}^{-1}$ stock solution, the suitable weight of the grinded powder was transferred from 10 tablets or the content of 10 capsules in a 100ml measuring flask and then made the volume up to the mark with Distilled water and (10ml from 0.1 M) hydrochloric acid solution. The resulting solution is filtered using the Whatman filter paper No. 1. Moreover, dilution was performed using the same solvent to obtain the desired concentration and the same procedure was performed as described in the general procedure [20].

2.6. Determination of maximum absorption wavelength

The Selenium complex UV-Vis spectrum was obtained at the wavelength of the greatest absorption 410nm as shown in Fig. 3.

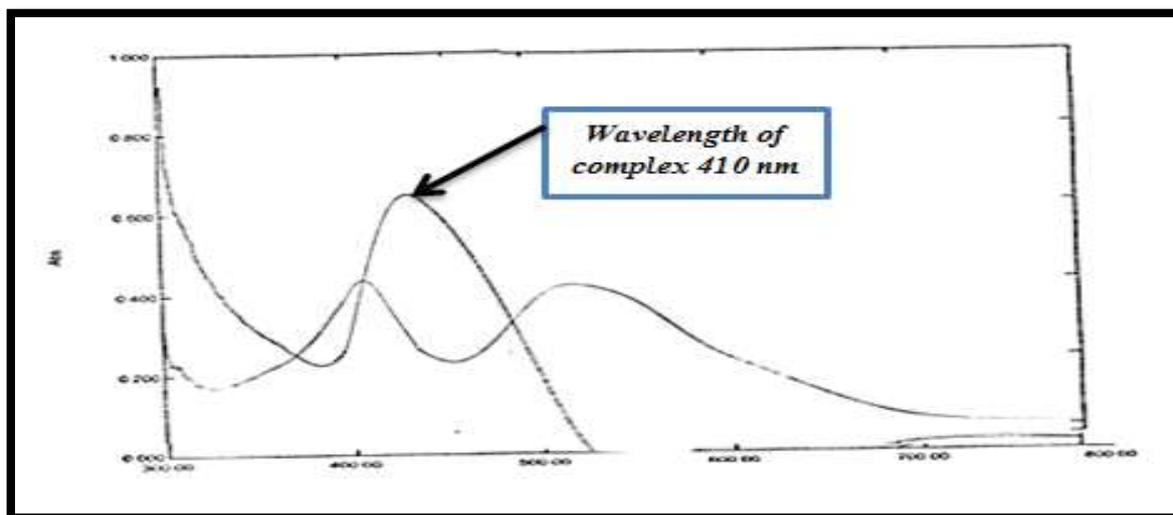
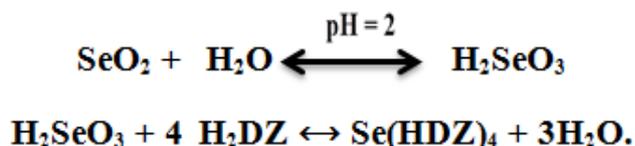


Fig. 3: UV-Vis Spectrum of: Selenium (IV) Complex in Acidic Medium (pH=2) with Se (IV) Conc. was $100 \mu\text{g mL}^{-1}$.

2.7. Results and Discussion

Selenium (IV) reacts with Dithizone (HDZ) in acidic medium and forms a Selenium-dithizonate complex, $\text{Se}(\text{HDZ})_4$ [21] according to the following equations:



2.8. Physical variables effect

Effect of flow rate

The change in peak height depends on the residence time of the sample zone in the system. The effect of the flow rate has been studied for Se (IV) complex, in order to get the highest peak over the range of 1.6 - 10.2 ml min⁻¹. The results showed that the peak height continued to increase with the flow rate until it reached 9.1 ml min in FIA and 3.6 ml min⁻¹ in SIA and then decreased with the increase in the flow rate as shown in Fig. 4.

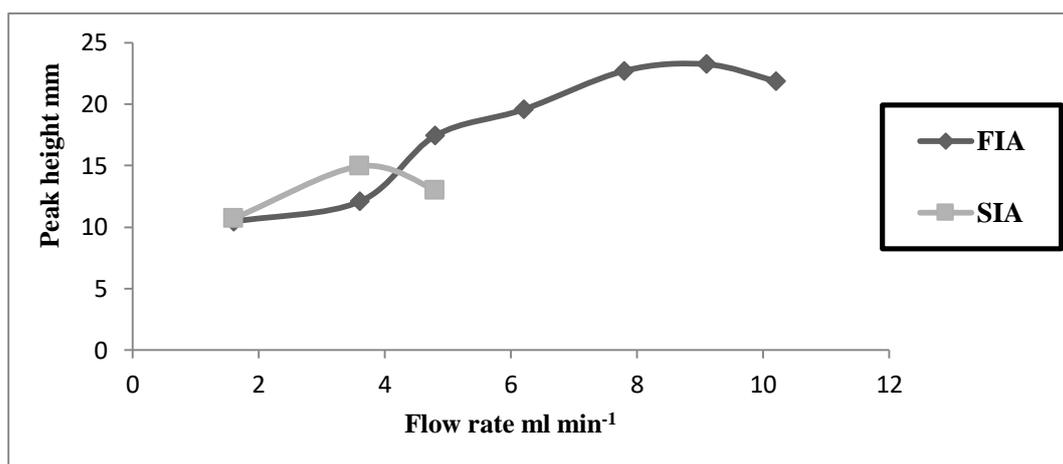


Fig. 4: Effect of Flow Rate on Se (IV) Complex.

Effect of reaction coil length

Reaction coil lengths of 10–30 cm were tested (Fig.5). The peaks became higher as the length of coil increased, but the additional increase did not result in any considerable change in sensitivity lengths, a reaction coil length of 15, 20 cm was chosen for FIA and SIA, respectively.

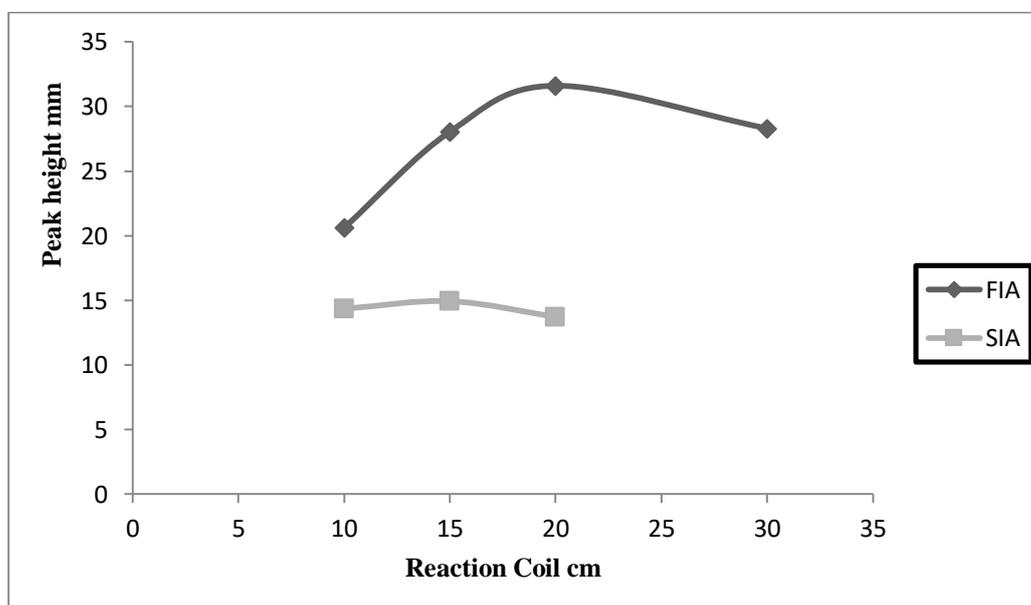


Fig. 5: Effect of reaction coil length on Se (IV) complex**Effect of Dithizone solution volume**

To get the best response the effect of reagent volume was also studied in the range 78.5–227.4 μL . A sample volume of 157 μl was chosen to FIA and SIA, respectively.

Effect of Se (IV) solution volume

Been studying The effect of Se (IV) solution volume on the peak height in the range of 78.5 to 227.4 μl . It was found that the highest peak was obtained at 157 μl for the FIA and SIA.

2.9. Chemical variables effect**Effect of Acid type as a medium for Se (IV) complex**

The acid effect was studied as a medium for complex formation. Hydrochloric acid, sulfuric acid and acetic acid were tested. It was found that the peak height of FIA and SIA was higher in a Hydrochloric acid medium.

Effect of reagent Dithizone Concentration

The effect of D_z concentration on the $\text{Se}(\text{HDZ})_4$ complex formation was investigated in the range of 0.001-0.05%, The highest peak was observed when the reagent concentration was 0.01% (w/v) was chosen to FIA and SIA, respectively.

Calibration curves, quantitation limits, detection limits and precisions

According to the optimum conditions, Se(IV) was determined and the calibration plots were prepared at 410 nm. The proposed method allows for the determination of 0.05-10 $\mu\text{g ml}^{-1}$, 0.05 - 5 $\mu\text{g ml}^{-1}$. Quantitation limits and detection limits were 0.096, 0.129 and 0.029, 0.039 for FIA and SIA, respectively, relative standard deviation RSD % for 10 replicate measurements at the concentration 10, 5 $\mu\text{g ml}^{-1}$ for FIA and SIA were 0.107 and 0.194. as shown in Fig. 6. Table 1 shows a comparison of the analytical parameters of the proposed two methods.

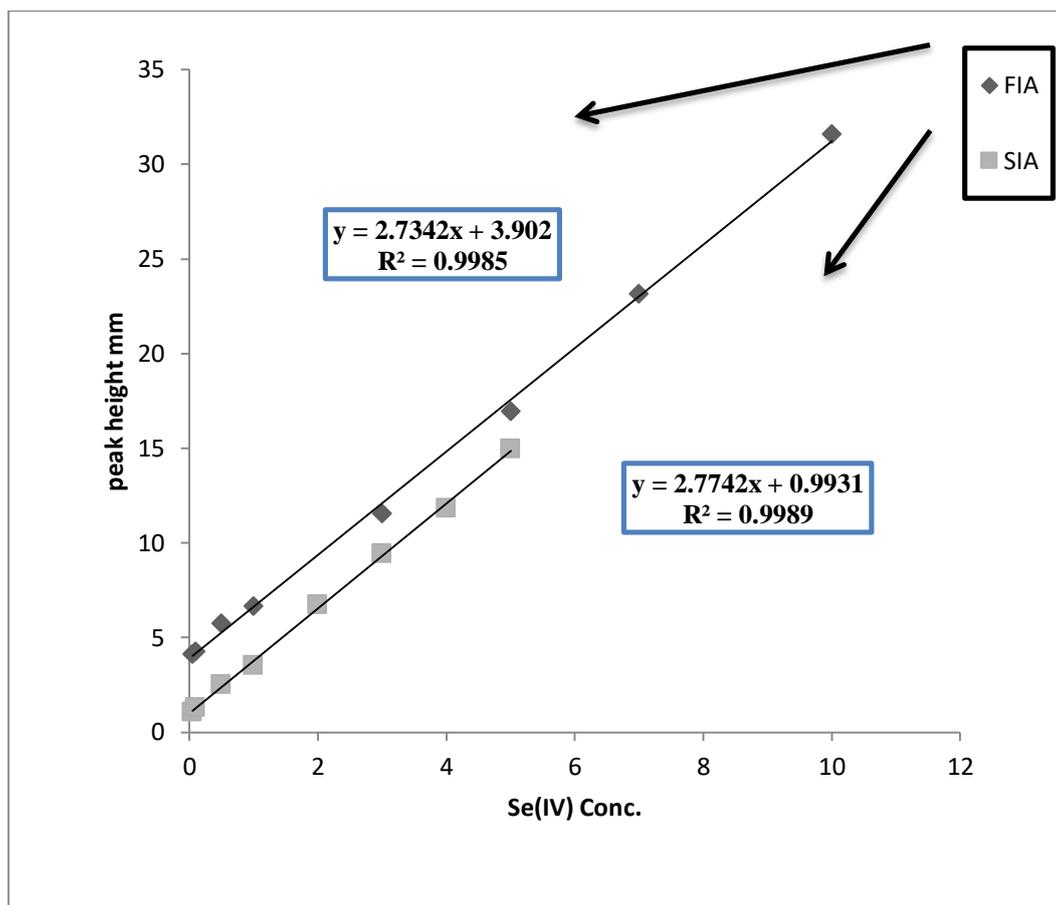


Figure 6: Calibration curves of Se (IV) complex (A) FIA (B) SIA with Dz.

Table 1: The Analytical Parameters of the Proposed Two Methods.

Parameter	FIA	SIA
Wavelength λ_{max} / nm	410	410
pH	2	2
linearity ranges $\mu\text{g ml}^{-1}$	0.05 - 10	0.05 - 5
Regression equation $Y = bx + a$	$Y = 2.7342x + 3.902$	$Y = 2.7742x + 0.9931$
Correlation coefficient r^2	0.9985	0.9989
Limit of detection / $\mu\text{g ml}^{-1}$	0.029	0.039
Limit of quantitation / $\mu\text{g ml}^{-1}$	0.096	0.129
RSD % n = 10	0.107	0.194
t-test*	0.2021	1.46
Recovery average %	94.110-103.676	90.72-102.24

Effect of Interferences

The effects of various anions and cations were studied such ($\text{Na}^+, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Ca}^{2+}, \text{Zn}^{2+}, \text{Fe}^{3+}, \text{Al}^{3+}, \text{Cl}^-, \text{CH}_3\text{COO}^-, \text{H}_2\text{PO}_4^-, \text{F}^-, \text{CO}_3^{2-}, \text{C}_2\text{O}_5^{2-}$ and SO_4^{2-}) on the determination of Se(IV) to find out the selectivity of the two designed methods. A known amount of interference has been added ($10 \mu\text{g mL}^{-1}$ and $50 \mu\text{g mL}^{-1}$) to Se(IV) solution of $10 \mu\text{g mL}^{-1}$. The results showed that Almost all anions and many cations used have no effect on the determination of Selenium in FIA and SIA methods.

Dispersion Coefficient

The dispersion coefficient (D) is a ratio of the concentration of the sample material before and after dispersion, the process is taken in that part of the fluid that produces the analytical reading, expressing:

$$D = H^0/H_{max}$$

Where H^0 : peak height without dilution outside the system, H_{max} : peak height relating with injected concentration inside the system. The results are in Table 2.

Table 2: Dispersion Coefficient Values

System type	Se concentration $\mu\text{g mL}^{-1}$	Response mm		Dispersion (D)
		H^0	H_{max}	$D=H^0/H_{max}$
FIA	5	23.67	16.963	1.395
SIA	5	20.05	14.953	1.340

Applications

The two design methods were applied to evaluate the analytical applicability of these methods; they were applied to the determination of Selenium (IV) in tap water and pharmaceutical samples respectively. The effectiveness of the method was confirmed by studying the recovery average, which varies from 94.110 to 103.676 and 90.72 to 102.24 for FIA and SIA, respectively as shown in Tables 2, 3.

Table 2: Determination of Selenium (IV) in Different Pharmaceutical Samples by Merging Zone Injection Method FIA.

Pharmaceutical sample	Taken value $\mu\text{g mL}^{-1}$	Found value $\mu\text{g mL}^{-1}$	E%	Recovery%*
Tap water	10	9.411	-5.890	94.110
VITACED, Switzerland	10	9.516	-4.840	95.160
Selenium Bonus, UK	10	9.443	-5.570	94.430
MERATRUM, Switzerland	5	5.184	3.676	103.676
BIOVITA, UK	5	4.878	-2.436	97.564
Antio_All, UK	5	4.739	-5.206	94.794

Table 3: Determination of Selenium(IV) in different Pharmaceutical Samples by Sequential Injection Method SIA.

Pharmaceutical sample	Taken value $\mu\text{g mL}^{-1}$	Found value $\mu\text{g mL}^{-1}$	E%	Recovery%*
Tap water	5	5.112	2.24	102.24
VITACED, Switzerland	5	4.536	-9.28	90.72
Selenium Bonus, UK	5	4.868	-2.64	97.36
MERATRUM, Switzerland	5	4.971	-0.58	99.42
BIOVITA, UK	5	4.891	-2.18	97.82
Antio_All, UK	5	4.937	-1.26	98.74

III.CONCLUSION

A new flow injection system and sequential injection were designed to determine Selenium (IV), study the best conditions for them and make a comparison between them. They were designed from materials that are available in the laboratory, inexpensive, and gave good results.

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