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Specific spectrophotometric method with trifluoroacetic acid for the determination of selenium(IV) in selenitetriglycerides

P. Suchocki [a,b,*], D. Jakoniuk [a], B.A. Fitak [a]

[a] *Department of Drugs Analysis, Warsaw Medical University, 1 Banacha Str., 02-097 Warsaw, Poland*
[b] *Department of Pharmaceutical Chemistry, Drug Institute, 30/34 Chelmska Str., 00-725 Warsaw, Poland*

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Abstract

The role of selenium as an antioxidant and anticancer agent is very well documented in the literature. Selenium compound showing the highest activity as a free radicals scavenger and as an anticancer agent should contain selenium at +4 oxidation level. The synthesis of selenitetriglycerides (named selol) was carried out in the Department of Drug Analysis at Warsaw Medical University (Polish Patent 1999). Selenitetriglycerides showed a dimeric structure. In a single dose toxicity studies performed in rats, LD50 was 100 mg Se kg^{-1} after oral administration of selol. The subcutaneous and intraperitoneal administration of selol showed extremely low toxicity. The aim of this work was to develop a new specific method for the determination of Se(IV) in selol. We stated that selenitetriglycerides react quantitatively with trifluoroacetic acid (TFA) in dichloromethane giving a red-coloured conjugate. However, recorded spectrum showed the maximum absorption in the wavelength 380 nm. The optimal conditions of the reaction were established, namely temperature 35 °C and reaction time 35 min. The reaction was proved to be specific because neither selenites nor other selol constituents react with TFA. The constructed calibration curve obeyed the Lambert–Beer law in the range of 0.1–7.4 mg ml^{-1}. Molar absorption coefficient is $\varepsilon = 9.46 \times 10^3$ l mol^{-1} cm^{-1} and $\varepsilon = 2.36 \times 10^5$ l mol^{-1} cm^{-1} calculated for selenium and selenitetriglyceride dimer (m.w. 1972.72), respectively. Obtained results for selenium determination were confirmed by AAS method. The developed method showed specificity and high sensitivity.

Keywords: Selenium; Selol; Selenitetriglycerides; Selenitetriglycerides analysis

1. Introduction

Selenium is an essential trace element for animals and humans. Its biological role was established following the discovery that selenium is a structural component of the active centre of many enzymes. Selenium is present as selenocysteine (Se-Cys) in at least 30 proteins, for example: glutathione peroxidase, selenoprotein P, selenoprotein W, type 1 iodothyronine deiodinase [1–4]. Selenium is regarded to be important for metabolic protection from oxidative stress, especially in diseases of the heart muscle and can also be important in protection against cancer [5–7]. Despite evident progress in cancer diagnosis and

* Corresponding author. Fax: +48-225-72-0735.
E-mail address: suchoc@amwaw.edu.pl (P. Suchocki).

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treatment, there is a constant need for a drug that could arrest premalignant process, thus reduce the risk of cancer. The role of selenium as an antioxidant and anticancer agent is very well documented in the literature. In addition, it has been proved that adequate concentration of selenium can restore the sensibility for chemotherapeutic agent of cytostatic-resistant malignant cells. Selenium compound showing the highest activity as a free radicals scavenger and an anticancer agent should contain selenium at +4 oxidation level.

Food contains organic forms of Se such as selenomethionine and selenocysteine but many experiments involving Se supplementation have used inorganic forms such as selenite. Absorption and use of organic and inorganic Se compounds differ. Organic forms appear to be re-used by the body more efficiently then inorganic forms, apparently because selenomethionine substitutes non-specifically for methionine residues in proteins but in such circumstances the Se has no bioactivity. For bio-activity and synthesis of specific selenoproteins, the trace element must be present as a selenide-like intermediate that incorporates into specific selenocysteine residues, usually at the active site of the selenoprotein. Evidence suggests that selenide is more-readily formed from inorganic rather than organic Se. Furthermore, selenite but not selenomethionine reacts readily with glutathione in erythrocytes to form selenodiglutathione, a compound that has anticarcinogenic properties and induces apoptosis of human tumour cells [8]. However, the main problem connected with the use of such compound (e.g. sodium selenite) is its high toxicity and high reactivity against some biologically active constituents of human organism. It was reported that LD50 of sodium selenite for rats after oral administration was 3.5 or 7.0 mg kg^{-1} of body weight [9,10]. The aim of our work was to find a compound containing selenium at +4 oxidation level and showing a low toxicity. The synthesis of selenitetriglycerides (named selol) was carried out in the Department of Drugs Analysis at Warsaw Medical University (Polish Patent 1999) [11]. We suppose that selenitetriglyceride in selol, similarly to triglyceride subject to β-oxidation in mitochondria. The acetyl-CoA and organoselenium rest containing Se(IV) are the products of enzymatic reactions. The synthesis comprised a two-step chemical reaction followed by the stage of purification. The sunflower oil (mixture of triglycerides) was oxidized with concentrated solution of $KMnO_4$ in 0.5% H_2SO_4. The emulsion was leave for separation by few days. Hydroxyl derivatives of triglycerides were esterificated with H_2SeO_3 in dioxane solution. The procedure involved the reaction of oxidation and esterification resulted in a very high yield. The tentative structure of selol is presented below: (Fig. 1). Until this synthesis has not been reported in literature but is described in (Polish Patent 1999) [11].

The biological studies on selol were performed in mice and rats. Selol was administered orally, subcutaneously and intraperitoneally at a single dose of 12 mg Se kg^{-1} [12]. Most of the data were obtained following oral administration as this is the proposed route in human patients. Concentrations of selol in the whole blood of rats were measured after 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h following the drug administration. The highest concentration of selol administered orally and subcutaneously was found out after 2 and 2.5 h, respectively. The obtained data indicate that absorption of selol from the rat gastrointestinal tract is high. Selol was also absorbed following subcutaneous and intraperitoneal administration [12]. The distribution profile of selol was investigated in rats after 0.5–8 h following its oral, subcutaneous and intraperitoneal administration. Selol was distributed in the whole body. The highest concentrations were found in the suprarenal gland, testes, epididymis, brain cortex and white matter of the brain. Lower concentrations were found in the cerebellum, liver, kidneys, lungs and spleen. A heart tissue contained the lowest selol concentration. These results were probably connected with a high lipophilicity of selol. A variability inter-animals was observed [12]. Following oral, subcutaneous and intraperitoneal administration of selol, metabolism products were eliminated from the body of the experimental animals primarily by renal excretion. The metabolism products of selol in urine, after microwave mineralization with 65% HNO_3 were determinated

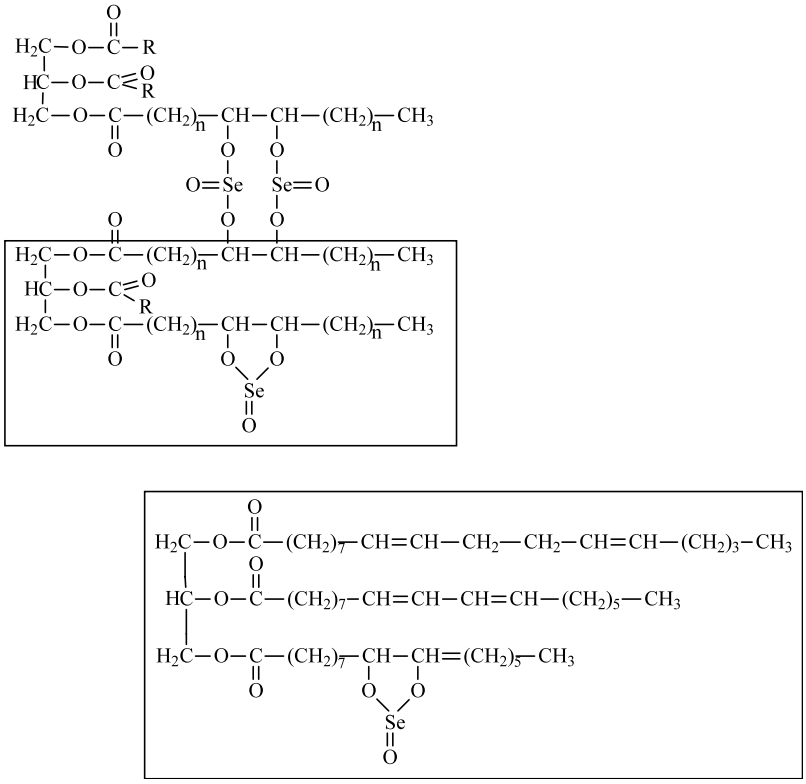

Fig. 1. A tentative structure of selol. The part of structure of selol majority to 5% Se(IV) is present in the frame (Octadeca-9,11-dienoic acid 1-[7-(5-non-3-enyl-2-oxo-2λ^4- [1,3,2] dioxaselenolan-4-yl)-heptanoyloxymethyl]-2-octadeca-9,13-dienoyloxy-ethyl ester). In higher concentrations—from 5 to 30% Se(IV), there is more dimer in selol (on the basis ^{1}H and ^{13}C NMR study).

as Se(IV) using AAS method. Elimination was completed within 24 h. Selenoenzymes activity like plasma glutathione peroxidase (plGSHPx) in rats and mice was high (unpublished data), but in the merino sheeps the activity of selenoenzymes (plGSHPx, cytosolic glutathione peroxidase (cGSHPx), type I and II iodothyronine deiodinases (ID-I, ID-II), and thioredoxin reductase measured in plasma, blood, and various animal tissues was not influenced in the adrenals, brain, kidneys, liver, pituitary gland, spleen and thyroid gland. The results indicate that the pharmacological action of selol is more complex than for 'classical' selenium containing compounds (Na_2SeO_3, Na_2SeO_4, SeO_2, selenocysteine, selenomethionine and seleno yeast) and further speciation study is necessary to clarify its biological functions [13]. In single dose toxicity studies performed in rats, LD50 was 100 mg Se kg^{-1} after oral administration of selol. The subcutaneous and intraperitoneal administration of selol showed very low toxicity, therefore precise determination was impossible. Selol did not exhibit a cumulative toxicity [14]. The effect of oral administration of selol on gross and behaviour was examined on mice. No visible changes were observed. Selol did not show a mutagenicity, what was proved with *Salmonella* strains (unpublished data).

The aim of this work was to develop a new specific method for the determination of Se(IV) as selenitetriglycerides contained in selol, which is an oil solution of selenitetriglycerides.

2. Experimental

2.1. Apparatus

A Shimadzu Model 2401 PC double-beam UV–visible spectrophotometer with 10 mm silica cuvette was used for all spectral measurements. The GBC Avanta Ultra Z automatic, single beam atomic absorption spectrometer, provided with an autosampler, a longitudinal Zeeman-effect background correction system, and a transversely heated graphite atomizer was used throughout. A CPI Int. selenium hollow cathode lamp 1.5 in.-Coded, operated at 10 mA was used for the determination of selenium at 196 nm with a 2.0 nm slit width. A Plazmatronika microwave mineralizer, model BM-1S, was used for mineralization of selol samples.

A Shimadzu HPLC chromatograph-detector UV/VIS type SPD-10AV was used.

2.2. Reagents and materials

Selenium atomic absorption standard solution in 1% HNO_3, 1020 μg ml^{-1} (Aldrich) was used for the preparation of selenium standards. Nickel(II) nitrate hexahydrate p.a. (Fluka) was used as a chemical modifier for selenium. Sixty five percent of nitric acid (VI) p.a. (Merck) was used for microwave method mineralization of selol samples. Doubly distilled, de-ionized water 18.3 MO cm^{-1} resistivity was used for preparation of solutions. 99.8% Trifluoroacetic acid (TFA) for spectroscopy (Merck); 99.8% dichloromethane for HPLC (LAB-SCAN); acetonitrile for HPLC (Merck).

2.3. Procedure

2.3.1. Determination of total content of Se(IV) in 2% selol by Zeeman electrothermal atomic absorption spectrometry

A 2% selol contain approximately 20 mg of Se(IV) in 1 ml of oil in form of selenitetriglycerides. 0.1 g of selol was exactly weighted and placed in to a teflon crucible together with 3 ml of 65% HNO_3 and heated in a microwave mineralizer according to the program (Table 1). When cool, it was diluted to 10 ml with water and transferred to 25-ml measuring flask. Then, this solution was diluted 1000 times in a few steps with a 5 mg ml^{-1} solution of $Ni(NO_3)_2$ in 1% HNO_3. Four samples were prepared in this way. Selenium determination was carried out using the AAS technique [15,16]. Selenium standard solutions—40, 100 and 200 ng Se ml^{-1}—were prepared in the same conditions as selol samples. Ten microliter of selenium standard or samples solutions were wet-injected in the graphite furnance. The time/temperature programme is shown in Table 2. Slow solution uptake and slow solution injection conditions were selected. The injection was carried out at 39 °C. Three standard additions with four replication each and peak height measurements were used for quantification. The results are shown in Table 3.

Table 1
Parameters of microwave mineralization

Step	Power (% max. energy = 135 W)	Time (min.)
1	20	1
2	30	1
3	40	2
4	50	2
5	75	2

2.3.2. Spectrophotometric determination of total content of Se(IV) in 2% selol in form of selenitetriglycerides with TFA in dichloromethane solution

2.3.2.1. Optimization of wavelength. A selol 2% was diluted with dichloromethane 2500 times. Then, 1 ml of 99.8% TFA was added to 4 ml of this solution. After 20 min of reaction in 35 °C the absorption spectrum of the reaction product was recorded in the range of wavelength 200–800 nm (Fig. 2).

2.3.2.2. Optimization of TFA concentration. To 4 ml of selol solution (like above) 0.2, 0.5, 1.0, 1.5 or 2 ml of 99.8% TFA was added. All samples were incubated in water bath at 35 °C for 50 min. After

Table 2
Furnance programme for selenium determination in selol samples using Ni as a chemical modifier

Stage	Temperature (°C)	Ramp (°C s^{-1})	Hold time (s)	Ar flow (l min^{-1})
Drying	110	10	10	3.0
	130	1.0	10	3.0
Ashing	800	10	10	3.0
	800	1.0	1.0	0.0
Atomising	2100	0.5	0.5	0.0
Cleaning	2300	0.1	0.9	3.0

Magnet field strength (T) = 0.70.

this time, maximum absorbance was obtained for sample, which contained 1 ml of 99.8% TFA.

2.3.2.3. Optimization of temperature. After 50 min of reaction in temperature over 45 °C Se(IV) in selenitetriglycerides has been reduced to $\downarrow Se^0$.

2.3.2.4. Optimization of reaction time in 35 °C. After dilution of selol 2% with dichloromethane to concentration 8.0 μg Se ml^{-1} to the test-tube with polished glass stopper 4 ml of this solution and 1 ml of 99.8% TFA was added. The mixture was transferred to a 1-cm quartz absorption cell and placed in spectrophotometer against the blank solution prepared in a similar way. An absorbance of the mixture was measured at 380 nm, at 5-min intervals, by 50 min in temperature 35 °C (Table 4).

2.3.2.5. Calibration curve. 0.1 g of 2% selol containing approximately 2 mg of selenium(IV) was accurately weighted into measuring flask and diluted 2500 times. Then 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 or 4.0 ml of this solution was transferred into 10 ml measuring flask. Thus obtained concentrations corresponded to: 0.1850, 0.5549, 0.9248, 1.8496, 2.7744, 3.6992, 4.6240, 5.5488, 6.4736 and 7.3984 μg ml^{-1} of selenium. The samples were completed to the 4-ml volume with dichloromethane. One milliliter of 99.8% of TFA was added to each tube. The tubes were loosely stoppered and heated for 35 min in 35 °C in a water bath. Afterwards, the tubes were cooled at 25 °C. The blank solution was prepared in the same way, but without selol. Absorbances of samples were measured at 380 nm, against the selol blank. Calibration graph was prepared by plotting absorbance against selenium concentration. Statistic of curve is presented in Table 5.

2.3.2.6. Spectrophotometric determination of Se(IV) in form of selenitetriglycerides. Ten milliliter of 2% selol samples were exactly weighted (± 0.0001 g) in the range of mass 0.02–0.07 g. The samples were diluted 250 times with dichloromethane in the measuring flasks. Then, 4 ml of each solution was transferred into test tubes with stoppers and 1 ml of 99.8% TFA was added. The blank solutions were prepared in the same way. The tubes were loosely stoppered, transferred into

Table 3
The results of estimation of selenium(IV) in selol 2% using atomic absorption spectroscopy

	Weighed sample (g)	Mean absorbance	Concentration of Se(IV) (μg ml^{-1})	Amount of Se(IV) in 100 g of selol x_i (%)
Sample 1	0.1021	249.5	0.08249	2.0198
Sample 2	0.1019	254.0	0.08384	2.0569
Sample 3	0.1014	250.2	0.08259	2.0362
Sample 4	0.1016	252.7	0.08341	2.0524

Statistic: $\bar{x} = 2.041$; S.D. = 0.0169; $S.D._{\bar{x}} = 8.440 \times 10^{-2}$; $\mu_{95} = 2.041 \pm 2.686 \times 10^{-2}$; R.S.D. = 8.269×10^{-3}; $V = 0.0285 \times 10^{-2}$. $S.D._{\bar{x}}$, standard deviation of mean; μ_{95}, level of confidence 95%; V, variance.

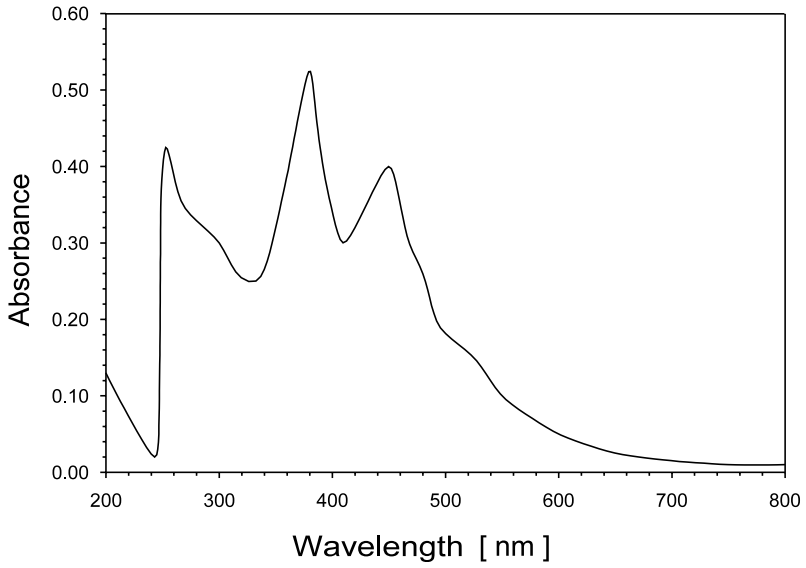

Fig. 2. UV–visible spectrum of selol sample after reaction with 99.8% TFA in dichloromethane solution.

water bath and heated for 35 min in a 35 °C. After this time absorbances of samples were measured at 380 nm. The results are shown in Table 6.

2.4. Preliminary chromatographic study

The 20 μl samples of sunflower oil (1 mg ml^{-1}) and 2% selol (0.1 mg ml^{-1}) in dichloromethane were separated using HPLC method. All analyses were carried out using SUPELCOSIL LC 18DB (250 × 4.6 mm) column at 30 °C, eluted with an dichloromethane–acetonitrile (4:1 v/v) mobile phase at 1 ml min^{-1} with detection by absorbance at 230 nm. The chromatograms are shown in Fig. 3.

3. Results and discussion

The aim of this work was to develop a new quantitative, analytically specific method for the determination of Se(IV) as selenitetriglycerides contained in selol which is an oil solution of the analyte. In our investigation it has been found using high-performance liquid chromatography, that selol is a mixture of more than 40 various

Table 4
The results of optimization of reaction temperature of selenitetriglycerides with TFA at 35 °C

Time (s)	Absorbance $\lambda = 380$ nm	Concentration (calculated with calibration curve) (μg ml^{-1})	$\log(C_0 - C_t)$
300	0.438	3.6678	0.4365
600	0.484	4.1167	0.3586
900	0.507	4.3412	0.3136
1200	0.520	4.4681	0.2860
1500	0.526	4.5266	0.2726
1800	0.528	4.5461	0.2681
2100	*0.529*	*4.5559*	*0.2658*
2400	0.528	4.5461	0.2681
2700	0.526	4.5266	0.2726
3000	0.525	4.5169	0.2749

Table 5
Statistic of curve for spectrophotometric determination of Se(IV) in selenitetriglycerides at 380 nm

Parameters of equation $A_{380} = (a \pm S_a)x + (b \pm S_b)$ in the range of concentration 0.18–7.39 μg ml^{-1}	
a	0.1025
b	0.0622
S_a	2.935×10^{-3}
S_b	3.022×10^{-2}
$S_{(x,y)}$	8.401×10^{-2}
r	0.9991

a, Slope; b, intercept; S_a, mean square error a; S_b, mean square error b; $S_{(x,y)}$, root-mean square error; r, correlation coefficient.

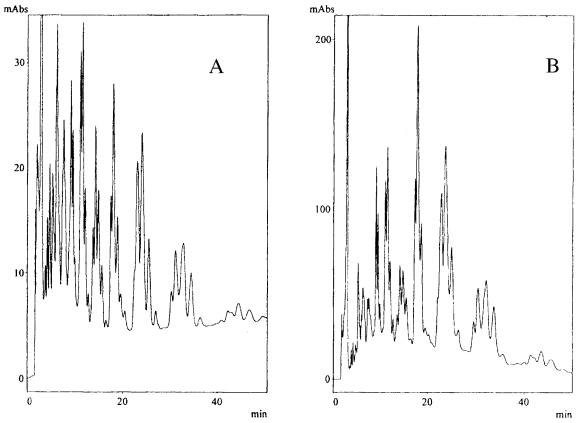

Fig. 3. HPLC analysis of 20 μl samples of triglycerides in dichlorometane (1 mg ml^{-1}) from sunflower oil (A) and selenitetriglycerides (0.1 mg ml^{-1}) in the same solvent from 2% selol (B). All analyses were carried out using SUPELCOSIL LC 18DB (250 × 4.6 mm) column at 30 °C, eluted with an dichloromethane–acetonitrile (4:1 v/v) mobile phase at 1 ml min^{-1} with detection by absorbance at 230 nm.

selenitetriglycerides (Fig. 3). The retention times of selenitetriglycerides and triglycerides are very similar, while absorbance of selenitetriglycerides in $\lambda = 230$ nm is about 100 times higher. We suggest that form of selenium(IV) in selenitetriglycerides react quantitatively with TFA in dichloromethane forming an red coloured conjugate, with good regression coefficient of correlation ($r = 0.9991$). The nature/structure of the red coloured conjugate formed is still unknown. The absorption spectrum of the reaction product (Fig. 2) suggests that the best analytical wavelength is that of 380 nm. Various parameters affecting the colour reaction of selenitetriglycerides with TFA were investigated. Experimental data show that maximum sensitivity at 380 nm is reached when concentration of TFA in dichloromethane is about 20%, temperature of reaction is 35 °C and time of reaction is 35 min, as described in the recommended procedure. The reaction was proved to be specific as neither selenites, selenates, selenocysteine, selenomethionine, seleno yeast nor other selol constituents react with TFA. The constructed calibration curve obeyed the Lambert–Beer law in the range from 0.1 to 7.4 mg ml^{-1}. Molar absorption coefficients are $\varepsilon = 9.46 \times 10^3$ l mol^{-1}

Table 6
The results of estimation of selenium(IV) in selol 2% using spectrophotometric metod with TFA in dichloromethane

No. of sample	Weighed amount of selol (g)	Mean absorbance	Concentration of Se(IV) in samples (μg ml^{-1})	Amount of Se(IV) in 100 g of selol x_i (%)
1	0.0619	0.485	4.124	2.083
2	0.0230	0.203	1.374	1.867
3	0.0558	0.437	3.657	2.048
4	0.0730	0.586	5.112	2.188
5	0.0307	0.252	1.852	1.885
6	0.0667	0.526	4.527	2.121
7	0.0426	0.326	2.575	1.889
8	0.0322	0.260	1.931	1.874
9	0.0521	0.412	3.414	2.048
10	0.0625	0.488	4.156	2.078

Statistic: $\bar{x} = 2.008$; S.D. = 0.1183; $S.D._{\bar{x}} = 3.550 \times 10^{-2}$; $\mu_{95} = 2.008 \pm 0.268$; R.S.D. = 5.590×10^{-2}; $V = 0.0140$. $S.D._{\bar{x}}$, standard deviation of mean; μ_{95}, level of confidence 95%; V, variance.

cm^{-1} and $\varepsilon = 2.36 \times 10^5\ l\ mol^{-1}\ cm^{-1}$ calculated for selenium and selenitetriglyceride dimer (m.w. 1972.72), respectively. Obtained results were confirmed by means of AAS method for selenium determination. The presented method appeared to be specific and sensitive enough for determination of selenium(IV) in selenitetriglycerides.

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