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How Safe are Antioxidant Food Supplements Containing Selenium?

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Abstract

Three antioxidant food supplements were analysed for selenium (Se) and its species. Sample A main constituents were coenzyme Q10, selenium (as medical yeast), vitamin C and natural vitamin E. The product is used for maintaining health and strengthening physical and mental abilities, stimulating the immune system, inhibiting the development of atherosclerosis, strengthening a weakened heart. Sample B main constituents were coenzyme Q10, selenium, vitamin E and beta-carotene. The product is advertised as high dosage natural coenzyme Q10, which provides supply of energy to all cells of human body. Sample C main ingredients were coenzyme Q10, selenium (as sodium selenite), beta-carotene and vitamin E with the same positive effects described as for the samples A and B. The samples were digested and analysed for Se content by hydride generation atomic fluorescence spectrometry (HG-AFS). For Se speciation, enzymatic hydrolysis with the enzyme protease was performed and soluble Se species was determined by ion-exchange chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The results showed that the value of Se obtained for one of the products (C) far exceeded the declared value; instead of 10 μg Se per capsule, 69 μg of Se per capsule was found. The declared Se species in the same product was sodium selenite, but only $SeCys_2$ was detected (55% of the total Se). In product B that did not have a declared Se form added, we detected 60% of total Se as selenite. In product A 75% of total Se was present as SeMet. It is worrying that the Se species determined and declared were different in two selected antioxidant food supplements.

Keywords: Antioxidant food supplements, selenium species, coenzyme Q10, stability, HPLC-ICP-MS

1. Introduction

Selenium as a trace element is essential for normal growth and development of the human organism.[1] Its biological functions are carried out by selenoproteins, several of which are constituents of oxidant defence enzymes.[2] Therefore, the nutritional need for selenium are worldwide a subject of current discussion.[3] Supplementation represents a sizeable portion of the multibillion euros nutritional supplement/functional food/animal business. During the last decade, the pharmaceutical market became overwhelmed with nutritional supplements based on antioxidants, where the coenzyme Q10 and selenium are widely discussed and advertised.[4–6] These antioxidants have the ability to enhance the immune system, effectiveness and memory, to reduce risk of cardiovascular diseases by lowering blood pressure and providing cellular protection, as well as protecting against specific cancers.[4,7] However to date, manufacturers often provide information only on total Se concentration, but no or little information on the Se species present in the supplements. Selenium may be added to food supplements (Directive 2002/46/ES, 2002) as inorganic Se (sodium selenate, sodium selenite and sodium hydrogenselenite), while Se in organic form may be added until the end of the year 2009.[8] Since selenium supplements contain selenium in different chemical forms, the question arises in which form Se should best be consumed. Ideally, Se should be supplemented in the form or forms in which it occurs in major staple foods. In the majority of supplements, the selenium is present as Se-methionine. However, in multivitamin preparations, infant formulas, protein mixes, weight-loss products and animal feed, sodium selenite and sodium selenate are predominately used.[9]

Stibilj et al. (2005) verified the advertised values of Se in food supplements, and discovered that 2 out of 14 supplements did not comply with the recommenda-

tions given in the 27th edition of the USA Pharmacopoeia, which states that minerals and vitamins in food supplements should be within 90 to 200% of the declared value.[10] B'Hymer and Caruso (2000) evaluated six different brands of yeast-based Se food supplements obtained from local stores in the USA.[11] All yeast-based Se food supplements were found to have near the labelled values based on total Se, and had reasonable uniformity in tablet to tablet content. Se-enriched yeast supplements have been widely studied by Dumont et al. (2006).[12] Gosetti et al. (2007) analysed six typical selenite based commercial diet supplements. They believed that side reactions are likely to occur among the different components of such formulations, therefore independent experiments were performed based on selenite and ascorbic acid, present in excess in these products. Analyses showed that only about 1 h after preparation, the signal of selenite was no longer present in detectable amounts.[13]

The easy access to supplements, also available in drugstores, makes their use uncontrolled and potentially dangerous. Coenzyme Q10 and selenium are the most popular antioxidants, therefore we decided to check whether the Se content in these antioxidant food supplements, which nowadays are flooding the market, are in agreement with the advertised values. Further, these supplements were analysed for the Se species present and the stability of these selenium species was investigated, since their benefits to human health are strongly correlated with the chemical form consumed.

2. Experimental

2. 1. Samples

Three antioxidant food supplements (namely sample A, sample B and sample C) were selected on Slovene market:

– Sample A main constituents were coenzyme Q10, selenium (as medical yeast), vitamin C and natural vitamin E. The product is used for maintaining health and strengthening physical and mental abilities, stimulating the immune system, inhibiting the development of atherosclerosis, strengthening a weakened heart. Four lots were taken, with an average capsule mass 0.98 g, taken as an average of 10 capsules. Product was present as soft gel capsules.

– Sample B main constituents were coenzyme Q10, selenium, vitamin E and beta-carotene. The product is advertised as high dosage natural coenzyme Q10, which provides supply of energy to all cells of our body. One lot was found on Slovene market during a half year period, with the capsule mass 0.81 g, taken as an average of 10 capsules.

– Sample C main ingredients were coenzyme Q10, selenium (as sodium selenite), beta-carotene and vitamin E with the same positive effects described as for the samples A and B. One lot was accessible on Slovene market during a half year period with an average mass of capsule 0.45 g, taken as an average of 10 capsules.

2. 2. Determination of Total Se Concentration

For the determination of total Se in the samples, digestion with HNO_3 (Merck, Suprapur), H_2SO_4 (Merck, Suprapur), H_2O_2 (Merck, p.a.) and V_2O_5 (Merck, p.a.) in H_2SO_4 was used, followed by reduction with HCl (Merck, Suprapur) and detection with HG-AFS (PS Analytical). The details of mineralization and the chemical and instrumental operating conditions (Table 1) were reported elsewhere.[14]

2. 3. Extraction and Speciation

The supplements were homogenized. In the case of soft gel capsules (A) extraction with diethyl-ether (Merck, p.a.) was performed to remove oils and fats. 1.5 g content of capsule was shaken with 12 mL of diethyl-ether. The mixture was centrifuged for 5 min at 5,000 rpm (5804R, Eppendorf). The soluble part was separated from the insoluble and the procedure was repeated three times more. Diethyl-ether was evaporated from the soluble part to dryness at room temperature. Both phases were analysed for Se content by HG-AFS (see above). The insoluble part was used for Se speciation analysis.

For Se speciation analysis, 8 g of water containing 60 mg of protease from *Streptomyces griseus* (type XIV: bacterial, 4.4 units/mg solid; Sigma) was added to 0.6 g of dry sample. The mixture was shaken for 24 h at 37 °C. After this extraction procedure, the extract was centrifuged at 11,000 rpm for 60 min at 4 °C. The supernatant was filtered through 0.45 and 0.22 μm Millex GV filters (Millipore Corporation) and subjected to selenium speciation analysis by HPLC-ICP-MS. Supernatants and sediments were stored at minus 20 °C until analysis for total Se by HG-AFS (Table 1). The rest of the parameters (enzyme purity, sample mass, incubation time) were published elsewhere.[15–17]

Sediments were digested and analysed for Se content as described above (determination of total Se concentration). For the determination of total Se in supernatants, digestion with HNO_3 and H_2O_2 was used. The next steps were reduction with HCl and detection by HG-AFS. A detailed description is given in the reference.[15] To check the accuracy and precision of the method for total Se analysis and analysis of Se species, a certified reference material (SELM-1, Selenium Enriched Yeast) was analysed simultaneously.

2. 4. Separation and Detection of Se Species

The concentrations of extracted Se species were determined using an ion exchange HPLC system (Agilent 1100, Waldbronn, Germany) coupled directly to ICP-MS (Agilent 7500ce, Tokyo, Japan). As standards, the Se species (Na_2SeO_3 (Se(IV), Sigma-Aldrich, >98%), Na_2SeO_4 (Se(VI), Sigma-Aldrich, SigmaUltra), Se-methionine (SeMet, Fluka Chemie, >99%), Se-cystine ($SeCys_2$, Fluka Chemie, >98%) and selenomethylselenocysteine (SeMeSeCys, Fluka Chemie, >98%) were used. The Se species were separated on an anion exchange column (Hamilton PRP-X 100) using gradient elution with aqueous mobile phase containing 3 and 10 mM citric acid (Fluka Chemie, puriss p.a.) in 2% methanol (pH 4.8, Primar, Fisher Scientific UK, trace analysis grade). Methanol was added to the mobile phase to increase the sensitivity of the selenium signal. While $SeCys_2$ is eluted with the void volume of the anion exchange column, a cation exchange column (Zorbax 300-SCX) was used with an aqueous mobile phase containing 3 mM pyridine solution (pH 2.1, Fluka Chemie, puriss p.a.) with addition of 2% methanol. Summarised operating conditions are presented in Table 1 and are reported in details elsewhere.[15] The chromatograms of Se species obtained under the optimal conditions are presented in Figure 1.

3. Results and Discussion

3. 1. Optimisation of the Extraction Parameters for Se Speciation

Some supplements selected were present as oils (product A). Therefore we tried to purify/separate fats from proteins in the soft gel capsules with diethyl-ether. Several clean-up steps were performed and the solution

Table 1: Optimal HG-AFS and HPLC-ICP-MS operating conditions for Se and its species determination.

Parameter	Value
HG-AFS	
instrument: PS Analytical	
carrier gas flow rate (mL/min)	1
argon gas flow rate (L/min)	0.26
nitrogen gas flow rate (L/min)	3
conc. of $NaBH_4$	1.4% w/v
conc. of HCl for HG	2 M
conc. of HCl in carrier	2 M
HPLC	
instrument: Agilent 1100	
Anion exchange chromatography:	
Hamilton PRP–X 100 column	4.1 mm × 250 mm × 10 µm
mobile phase A	3 mM citrate buffer in 2% MeOH (pH 4.8)
mobile phase B	10 mM citrate buffer in 2% MeOH (pH 4.8)
gradient	14 min gradient from 100% A to 50% A; 1 min gradient to 100% B, isocratic to 25 min; gradient for 2 min to 100% A; isocratic to 32 min
flow rate (mL/min)	0.5
injected volume (µL)	50
Cation exchange chromatography:	
Zorbax 300–SCX column	4.6 mm × 250 mm × 5 µm
mobile phase	3 mM pyridine solution in 2% MeOH (pH 2.1)
flow rate (mL/min)	0.5
injected volume (µL)	50
ICP-MS	
instrument: Agilent 7500ce	
nebulizer: Micro Mist	
plasma:	
RF power (W)	1500
outer gas flow rate (L/min)	15.0
octopole reaction cell:	
H_2 gas flow rate (mL/min)	4.0
measuring parameters:	
m/z monitored	^{78}Se
integration time (s)	0.3

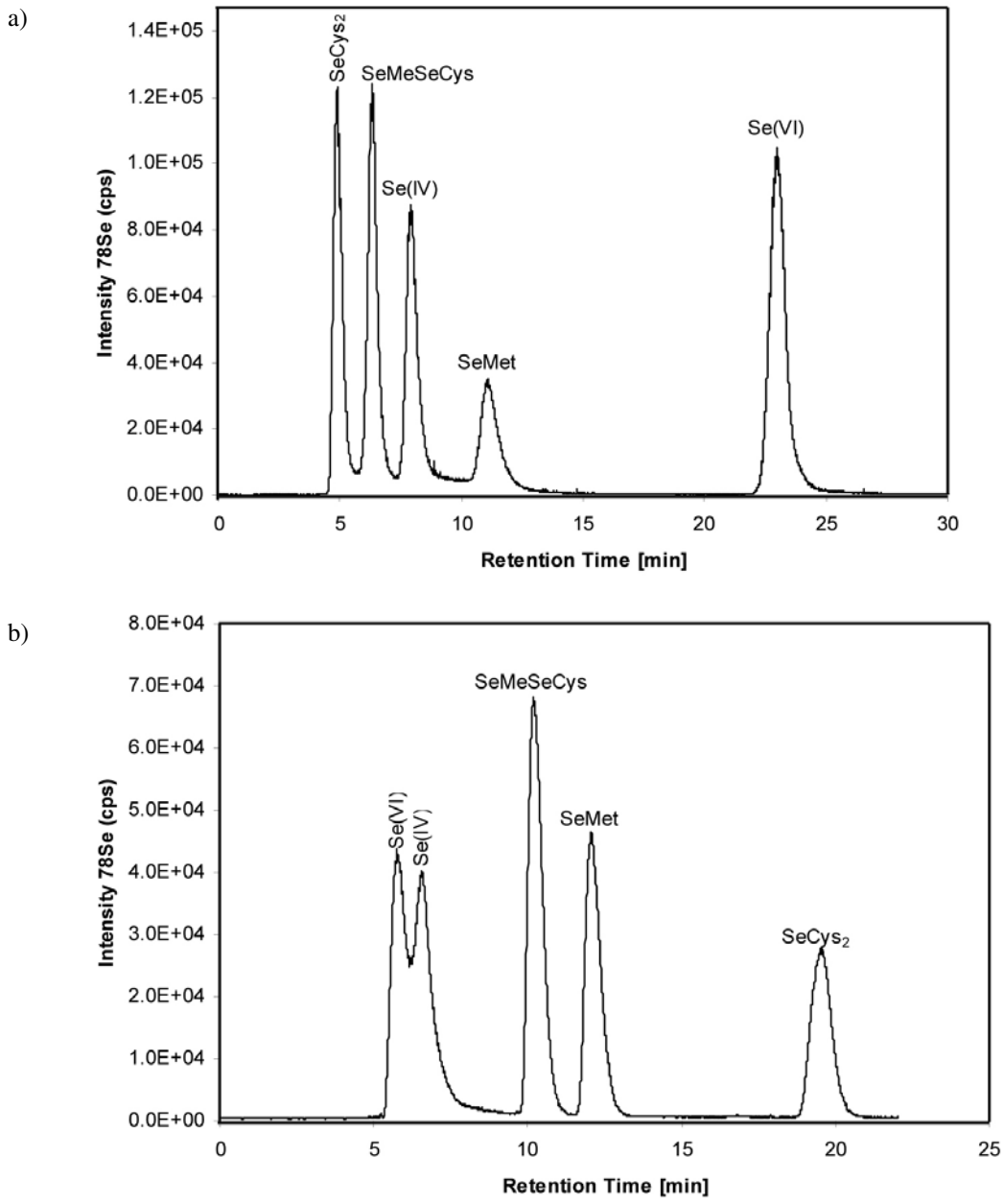

Figure 1: Chromatogram of a mixture of Se species with mass fractions of Se around 100 ng/g solution each on anionic (a) and cationic (b) exchange columns.

and residue obtained were analysed for Se content. In a one-step extraction we were not able to quantitatively remove fats, therefore we repeated the extraction. For almost quantitative elimination of fats, four extractions were required. Afterwards, 96% of total Se was present in the insoluble protein fraction, which was taken for Se speciation analysis (Table 2). In the fat fraction only 2% of total Se remained.

Extraction efficiency for Se speciation analysis depends upon the nature of the sample and the extraction conditions. In our study, two different types of extraction were used for Se species from capsules. The first was simple water extraction for water-soluble Se species, and the second was enzymatic hydrolysis to release Se species bound to proteins.

In products A and C the efficiency was not satisfying (<10%) when extraction of Se species was performed in water alone. So, due to the fact that Se in antioxidant food supplements may be bounded to proteins the simple extraction conditions, MilliQ water as extraction solvent and single step extraction, were also used with enzymatic hydrolysis. Only the mass of enzyme was considered. 60 or 120 mg of the enzyme protease XIV in 8 mL of MilliQ water was added to 600 mg of sample. Solutions

Table 2: Agreement between declared and determined Se values and the species present (µg Se/capsule for product A, B, C and µg Se/g sample for CRM).

product	Se content declared value	determined value	Speciation analysis extraction with diethylether soluble	Speciation analysis extraction with diethylether insoluble	enzymatic extraction soluble	enzymatic extraction insoluble	declared Se species added	determined Se species
A	15	14.4 ± 0.9	0.3 ± 0.1	13.8 ± 0.2	12.2 ± 0.9	1.4 ± 0.2	Se as medical yeast	75 % of total Se/ capsule (87 % of soluble Se/ capsule) as Se-methionine
B	10	9.4 ± 0.5	–	–	5.8 ± 0.1	3.7 ± 0.1	Not stated	60 % of total Se/ capsule (97 % of soluble Se/ capsule) as selenite
C	10	69.1 ± 5.0	–	–	40.4 ± 2.0	27.8 ± 1.9	Se as sodium selenite	55 % of total Se/ capsule (93 % of soluble Se/ capsule) as Se-cystine
CRM: **SELM - 1** (Selenium Enriched Yeast)	2059 ± 64 *	2089 ± 142	–	–	1763 ± 8	322 ± 41	3431 ± 157 * as Se-methionine (67 % of total Se)	69 % of total Se (81 % of soluble Se) as Se-methionine

Results are reported as an average of three determinations along with the standard deviation. * certified values

were then incubated for 24 h at 37 °C. The efficiency increased to an average of 60% for products B and C and to 85% for product A (Table 2) in the presence of enzyme, irrespective of its mass.

3. 2. Stability of Se Species

One of the possible reactions affecting the stability of supernatants during extraction and storage is reduction of Se species, mainly inorganic Se. According to the literature, selenite is easily reduced to Se(0) by ascorbic acid.[13,18] In the present study, the possibility of reactions taking place among the different components present in the antioxidant food supplement C (coenzyme Q10, beta-carotene and vitamin E) and inorganic Se was studied.

An enzymatic solution of Se(IV) and Se(VI) with a mass fraction of Se around 100 ng/g was used in a test experiment with a) coenzyme Q10 (Sigma-Aldrich, coenzyme Q10 : selenium = 1 : 1); b) beta-carotene (Carl Roth, beta-carotene : selenium = 1 : 10); c) vitamin E (Fluka, vitamin E : selenium = 1 : 2); d) coenzyme Q10, beta-carotene and vitamin E (coenzyme Q10 : Se : vitamin E : beta-carotene = 10 : 10 : 5 : 1, w/w). The ratio between ingredients was taken as present in the supplement C. After mixing inorganic Se with the other ingredients and hydrolysis, Se speciation analysis (performed immediately) showed that about 3% of Se(IV) was oxidised to Se(VI), regardless of the ingredient combination used. Results obtained for Se(VI) showed no transformation.

Further, thermal stability of inorganic Se in the presence of coenzyme Q10 (B.M.P. Bulk, Medicines & Pharmaceuticals) was investigated. Se(IV) or Se(VI) and coenzyme Q10 were dissolved in a solution of ethanol and water (20 : 1, v/v) and heated at 60 °C and/or 80 °C for 10 min. The solutions were evaporated to dryness at room temperature. Hydrolysis was performed without and with the enzyme protease XIV and the supernatant analysed for Se species present. The results showed that about 3% of Se(IV) was oxidised to Se(VI), regardless of the temperature used, and no new Se species was present in the chromatogram obtained.

Moreover, to see if there was any change in Se composition we left the mixtures overnight at room temperature and repeated the analysis. No changes were observed within 24 h. Therefore, the main ingredients in the products do not affect the content and transformation of inorganic Se species.

3. 3. Verification of the Method

In most such dietary supplements the predominant chemical form of Se is SeMet, either as synthetic L-SeMet or yeast-based SeMet. There is lack in availability of certified reference materials for Se species contents whit which to evaluate measurement performance for SeMet. In response to this need, the Institute for National measu-

rement Standards (INMS) of the National Research Council Canada (NRC) recently completed certification of a new selenized yeast material (Selenium Enriched Yeast, SELM-1), certified for SeMet, Met and total Se amount content.[14] There are currently no other certified reference materials available for quality control of measurements characterizing these supplements. The challenge in determining SeMet is that it is basically an amino acid measurement. Several extraction procedures were performed, enzymatic extraction as well as hydrolysis with methanesulfonic acid, leading to comparable results.[19] Nevertheless, variability between results obtained for SeMet in seven laboratories (from 1.970 to 3.587 mg/kg) is not negligible what proves how difficult it is to obtain accurate results in Se speciation analysis. Therefore, the accuracy of Se and its species determination using the method developed was checked by analysing the certified reference material SELM-1. A very good agreement for SeMet and total Se was found between our results and the values reported (Table 2).

3. 4. Se content and its Species in Antioxidant Food Supplements

Se values obtained for products A and B were in agreement with the advertised values (Table 2), the avera-

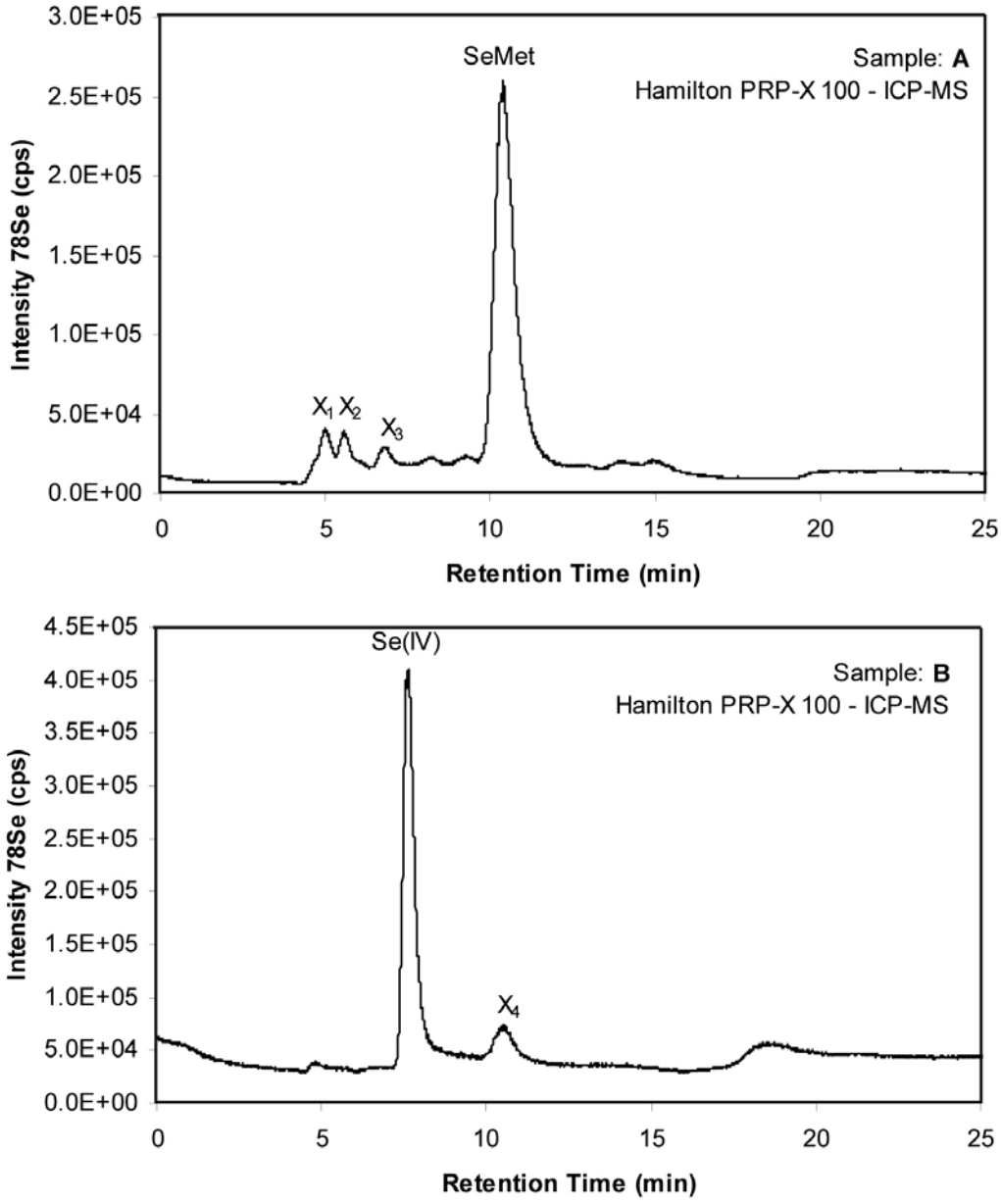

Figure 2: Chromatograms of enzymatic food supplement extracts obtained after separation on an anion exchange column (Hamilton PRP-X 100) using ICP-MS as the detection system. X_1–X_4 … unknown Se species

ge Se content was a little below the declared value but within experimental error (4% below the advertised value for product A and 6% below the advertised value for product B). The value of Se for obtained product C far exceeded the declared value: instead of 10 μg Se per capsule, 69 μg of Se per capsule was found. The recommended dietary intake for Se is 30–70 μg Se per day, while the tolerable upper intake level for adults is set at 400 μg Se per day.[20] Due to the fact that the Se content in this product exceeded the advertised value sevenfold, it represents a potential risk of Se poisoning, particularly when several capsules are daily consumed along with other food rich in Se. To the authors' knowledge, to date there have been no studies made on the Se content in food supplements containing antioxidants. Research was restricted mainly to multi-micronutrient supplements containing vitamins and minerals.[10, 11] In recent years there have been several cases of Se poisoning. Clark et al. (1996) reported a case of intoxication caused by a nutritional supplement. Although the advertised value of Se per six tablets was 5 μg, laboratory analysis demonstrated that the amount of Se per tablet was between 500 and 1000 times the declared amount.[21] Moreover, thirteen people were poisoned with tablets containing a 180-fold higher Se content than advertised.[22]

Suppliers often provide information on total Se content in products, but little or no information on the Se species. Since it is important to know the identity of the compounds the Se species present were investigated (Table 2, Figure 2, 3)). It is stated that Se was added to product A as medical yeast. We found about 75% of total Se in product A as SeMet. In product B, which does not have a declared Se form added, we detected 60% of total Se as inorganic selenium, as selenite. The declared Se species in product C was sodium selenite, but on doing speciation analysis it was not found in the extracts obtained. We were able to determine Se-cystine, which came with the void volume on the anion exchange column. To confirm this species separation on a cation exchange column and standard addition was made (Figure 3). About 55% of total Se corresponded to Se-cystine. Interferences between supplement ingredients were investigated (see section: Stability of Se species) but no effect on inorganic Se was observed. As a matter of fact, if reactions had taken place among the components during product preparation and storage of the capsules, it would be difficult to say in which form Se is present in the capsules.

From figures 2 and 3 it is seen that some other Se species were present in the extracts obtained. But, unfortunately we were not able to identify them, due to the fact that there is still lack of commercially available Se compounds. Moreover, the problem of identifying Se species could be important since their effects on human health still remain unknown. In our case, unidentified selenium species presented above 5% of the main soluble Se species determined. The major Se species identified in all products represented the quantitative amount of soluble Se. In sample A, 87% of soluble Se per capsule (75% of total Se/ capsule) was present as SeMet; in sample B, 97% of soluble Se per capsule (60% of total Se/ capsule) was in the form of selenite and in the sample C, 93% of soluble Se per capsule (55% of total Se/ capsule) as $SeCys_2$. It is worrying that Se species determined and their concentrations were different in each of the three selected antioxidant food supplements as well as the deviation from the

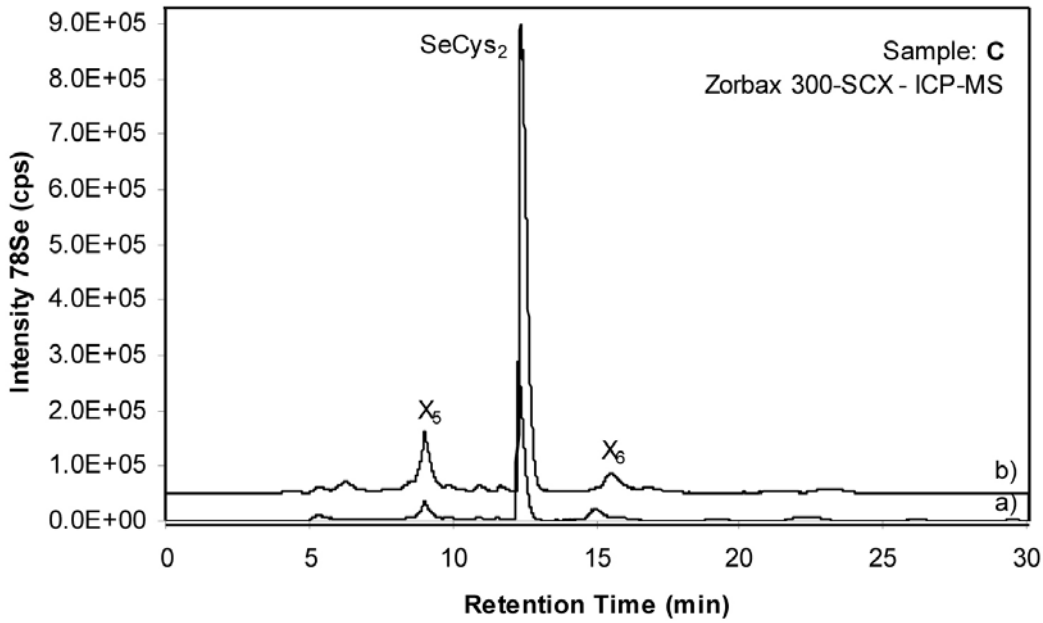

Figure 3: Chromatogram of enzymatic food supplement extract C obtained after separation on a cation exchange column (Zorbax 300-SCX), (a). To form a better diagram the chromatogram obtained on a cation exchange column for sample C with $SeCys_2$ addition was transposed by 0.5×10^5 cps upwards (b). X_5, X_6 … unknown Se species

declared levels is worrisome, although. B'Hymer et al. (2000) indicated that labelling of yeast-based food supplements containing Se-methionine may often be inaccurate, since of the six brands of common yeast-based Se food supplements, only two showed significant levels of selenium in the form of Se-methionine.[11] Further, Gosetti et al. (2007) analysed commercial diet supplements and two out of six products did not have Se species present (selenite) in the supplement as declared.[13]

4. Conclusions

Se values obtained for products A and B were in agreement with the advertised values, while on the other hand, the value of Se obtained for product C far exceeded the declared value: instead of 10 μg Se per tablet, 69 μg of Se per tablet was found and therefore supplement C represents a potential risk of Se poisoning. In the products analysed, the Se species added was declared in one out of the three products, namely product C (Se as sodium selenite). To product A Se was added as medical yeast. About 75% of total Se in product A was present as SeMet. In product B that does not have a declared form of Se added, we detected 60% of total Se as inorganic selenium, as selenite. The declared Se species in the product C was sodium selenite, but on doing speciation analysis it was not found. About 55% of total Se corresponded to Se-cystine. However, the main ingredients in product C (coenzyme Q10, beta-carotene, vitamin E), as well as temperature (60 °C or 80 °C), incubation during enzymatic hydrolysis (24 h, 37 °C) and/or overnight storage do not affect the presence and transformation of inorganic Se species. Thus, each brand had dramatically different profiles for the chemical form of Se present in the supplement. Regarding these results, the overall production of food supplements urgently needs control of Se and its species content (quality and quantity control) to assure the safety and quality of these products for the consumer's health.

5. Acknowledgements

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Povzetek

Določili smo vsebnost selena (Se) in njegovih spojin v treh prehranskih dopolnilih, ki vsebujejo antioksidante. Dopolnilo A vsebuje koencim Q10, Se v medicinskem kvasu, vitamin C in naravni vitamin E in se uporablja za okrepitev fizičnih in mentalnih sposobnosti, spodbudo imunskega sistema, za zaviranje razvoja ateroskleroze ter za okrepitev oslabljenega delovanja srca. Dopolnilo B vsebuje koencim Q10, Se, vitamin E in beta karoten in je oglaševan kot produkt z visoko vsebnostjo naravnega koencima Q10, ki zagotavlja dovolj energije za vse celice človeškega telesa. Sestavine dopolnila C so koencim Q10, natrijev selenit, beta karoten in vitamin E. Oglaševan je z istimi pozitivnimi vplivi na zdravje kot sta oglaševana produkta A in B. Vsebnost Se v vzorcih je bila določena po razkroju z metodo hidridne tehnike atomske fluorescenčne spektrometrije (HG-AFS). Za določitev Se spojin smo uporabili encimsko hidrolizo s proteazo. Separacijo in detekcijo topnih Se spojin smo naredili s tekočinsko kromatografijo v povezavi z masnim spektrometrom z induktivno sklopljeno plazmo (HPLC-ICP-MS). Vsebnost celokupnega Se v enem izmed analiziranih prehranskih dopolnil (C) je presegla deklarirano vsebnost za sedem-krat; namesto 10 μg Se na kapsulo smo določili 69 μg Se na kapsulo. Deklarirana Se spojina v tem dopolnilu je bila natrijev selenit, medtem ko smo z našimi analizami določili le Se v obliki SeCys2 (55 % vsebnosti Se). Dopolnilo B, ki je imelo samo deklarirano vsebnost elementa, je vsebovalo 60 % Se v obliki selenita. V dopolnilu A smo 75 % Se določili v obliki SeMet. Zaskrbljujoče je, da se določene in deklarirane vrednosti za Se spojine ne ujemajo v dveh izbranih prehranskih dopolnilih.