sciendo

*Polish Journal of Chemical Technology, 20, **4**, 84—87, 10.2478/pjct-2018-0058*

Stability of selenium diet supplement

Joanna Grzechulska-Damszel*, Agata Markowska-Szczupak, Antoni W. Morawski

West Pomeranian University of Technology Szczecin, Faculty of Chemical Technology and Engineering, Institute of Inorganic Chemical Technology and Environmental Engineering, Pułaskiego 10, 70-322 Szczecin, Poland
**Corresponding author: e-mail: joanna.grzechulska@zut.edu.pl*

Selenium is one of the trace elements playing a crucial role in a proper function of living organisms. Selenium intake varies, largely based on the selenium content of food. The daily Se intake recommended for adults amounts to 55 μg per day. The mean level of selenium in the population varies considerably between countries. Recent studies on the selenium dietary intakes in Poland showed that it is not sufficient to meet the daily requirement for this microelement so it is strongly recommended to employ selenium supplementation.
The commercial product SEL-BRCA1® being a selenium diet supplement was subjected to chemical and microbial analysis to determine its stability in storage time and conditions. Basing on the obtained results it can be stated that the stability of studied supplement, stored in the closed bottles, can be confirmed for the period of time up to 45 months after the production date and it is not recommended to store opened bottles for the period of time longer than 12 months. The studied supplement showed a very high microbial stability what underscores its usefulness as human medicines.

Keywords: selenium supplementation, chemical analysis, microbial analysis, stability.

INTRODUCTION

Trace elements play a crucial role in the maintenance of immune-endocrine, metabolic and cellular homeostasis[1]. One of them is selenium[2–8]. Selenium (Se) is a micronutrient which was discovered in 1817 by the Swedish chemist and physician Jöns Jacob Berzelius[9] in the sludge of leaden chambers used for the production of sulfuric acid. Although toxic in large doses, selenium is an essential trace mineral in the animal diet and in some plants. The biological role of selenium is associated with selenoproteins involved in the antioxidant defense system, animals thyroid hormones metabolism and spermatogenesis[10, 11].

There is a range of serum selenium levels associated with the lowest rate of all causing mortality – the levels should be maintained at an optimal level and be neither too low nor too high[4, 6, 8]. The total content of Se in the human organism shows wide variations depending on the population studied, the diet and the geographical area, while intake varies with the composition of the soil. Selenium intake varies, largely based on the selenium content of food. The mean level of selenium in the population varies considerably between countries. Thus, for some countries selenium supplementation should be considered, whereas for others it could be contraindicated.

According to some epidemiological data, low selenium dietary intake may be associated with higher cancer incidence and is also implicated in cardiovascular diseases, cirrhosis, diabetes and asthma[10, 12].

Recent meta-analyses indicate unequivocally that selenium supplementation of people with low initial serum selenium levels decreases the incidence of cancer by ~35% and of cancer mortality by almost 50%[12]. Recent studies on the selenium dietary intakes in Poland[11] showed that it is not sufficient to meet the daily requirement for this microelement so it is strongly recommended to employ selenium supplementation. The daily Se intake recommended for adults by the Scientific Committee on Food (SCF) of the European Commission amounts to 55 μg per day[13].

It is important for the selenium diet supplements to contain selenium ion as its tetravalent form since it is assimilated by living organisms in contradistinction of hexavalent form of selenium ions.

The aim of presented studies was to determine chemical and microbial stability of selenium diet supplement over a long period of storage time.

MATERIAL AND METHODS

The object of studies was a commercial product SEL-BRCA1®. This product is an unique composition of selenium designed for effective protection against selenium deficiency in Poland. It contains selenium in the form of an alcoholic solution of sodium selenite and is applied as selenium supplement for daily diet. It is prescribed to use two drops a day what gives 94.5% of recommended daily intake of selenium. It cannot be used as substitute of diversified diet. The bottle contains 15 ml of the supplement and suffices for 6 to 12 months.

Speciation analysis of Se(IV)/Se(VI) ions were conducted using ion chromatograph IC 850 Professional (Metrohm) equipped with MSM and MCS suppressors and conductometric detector. Analysis were performed using the column Metrosep A Supp 5 (250 mm long, inner diameter – 4.0 mm, column packing – polyvinyl alcohol with quaternary ammonium groups, particle diameter – 5 μm) connected with precolumn A Supp 4/5 S Guard. The mixture of sodium carbonate (POCh, Poland) and sodium bicarbonate (POCh, Poland) was used as an eluent (isocratic flow, flow rate – 0.7 ml/min). The solution of sulfuric acid (Sigma – Aldrich) was used for suppressor regeneration. The working temperature of column was 30°C. Presented optimal conditions of analysis allowed to obtain satisfactory speciation of Se(IV) and Se(VI) ions.

The series of standard solution were prepared using Na_2SeO_3 (Sigma – Aldrich) and Na_2SeO_4 (Sigma – Aldrich) as a source of Se(IV) and Se(VI) ions, respec-

tively. Basing on the obtained calibrating curves, the concentration of selenium ions in analyzed samples was determined. All reagents were analytically pure. The solutions were prepared in ultrapure water.

The microbial stability of selenium supplements of SEL-BRCA1® and placebo was tested by own method based on method developed by the The International Pharmacopoeia (Farmakopea Polska VI, 2002. Polskie Towarzystwo Farmaceutyczne)[14]. The microbial tests were carried out under aseptic conditions in tree replications once a month for two year. The following liquid media were used: Soyabean Casein Digest (pancreatic digest of casein 17.0 g, papaic digest of soyabean meal 3.0 g, sodium chloride 5.0 g, K_2HPO_4 2.5 g, glucose 2.3 g in 1 liter of distilled water, pH 7.3 ±0.2); Thioglycollate Broth (L-cystine: 0.5 g, sodium chloride 2.5 g, yeast extract 5.0 g, glucose 5.0 g, peptone K 15.0 g, sodium thioglycollate 0.5 g and agar 0.75 g in 1 liter of distilled water, pH 7.1 ±0.2). The solid media were obtained by supplementation with 15.0 g Agar-Agar (BTL, Poland). The media were prepared according manufacture's (BTL, Poland) instructions and distributed to test 10 ml test tubes. The vents of test tubes were closed by cotton wool and then sterilized in autoclave high-pressure saturated steam at 121°C for around 15–20 minutes. The media were incubated for 14 days. If no growth of microorganisms occurred in tests tubes, the media were considered to be sterile. 1 ml of SEL-BRCA1® and placebo were placed in aseptic conditions to six tubes (for each tested variant) and once again were plugged by cotton wool. The test tubes were placed in the incubator at 25°C and 37°C for 24 h. 0.3 ml of solution was then taken from each test tube and placed on appropriate solid media Soyabean Casein Digest Agar or Thioglycollate Agar. The Petri dishes were incubated at 37°C or 25°C, respectively. The bacteria colony were counted. Additionally, pathogenic bacteria tests were conducted by commercial POLCARGO INTERNATIONAL SP Laboratory (Szczecin, Poland). The following parameters were determined: the presence of *Salmonella* spp., the presence of *Escherichia coli*, the presence of coagulase-positive staphylococci, the number of yeast and molds and the total bacteria count TBC at 30°C.

RESULTS

Application of IC method using described above parameters resulted in a good speciation of SeO_3^{2-} / SeO_4^{2-} ions (Fig. 1). An example chromatograms of selenium supplement samples are presented in Fig. 2.

IC analysis results are shown in Table 1. It presents the concentration of SeO_3^{2-} / SeO_4^{2-} ions in particular

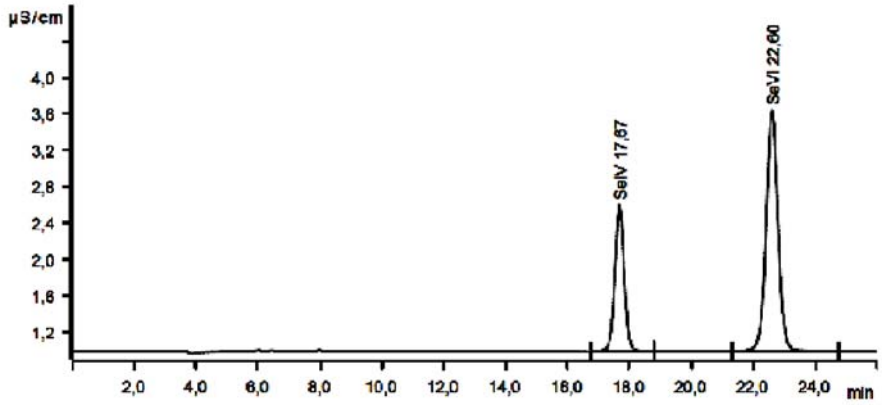

Figure 1. Speciation of SeO_3^{2-} / SeO_4^{2-} ions by IC

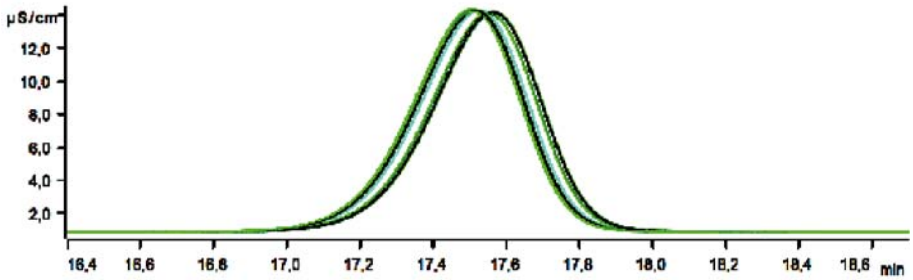

Figure 2. An example chromatograms of selenium supplement samples

Table 1. The concentration of SeO_3^{2-} / SeO_4^{2-} ions in particular samples, depending on the lot number and validation date

	Name	Lot number	Validation date	SeO_3^{2-} [mg/dm^3]	Se^{4+} [mg/dm^3]	SeO_4^{2-} [mg/dm^3]
1	SEL-BRCA1®	1X072	October 2012	1396.90 1396.71	869.15 869.03	–
2	SEL-BRCA1®	010412	April 2013	1377.05 1378.75	856.80 857.86	–
3	SEL-BRCA1®	011112	November 2013	1389.97 1392.21	864.84 866.23	–
4	SEL-BRCA1®	010513	May 2014	1395.40 1397.02	868.22 869.23	–

samples, depending on the lot number and validation date.

The obtained results showed that there was no significant differences in concentration of SeO_3^{2-} ion in the analyzed samples (Fig. 3). The concentration of SeO_4^{2-} ion was below the limit of sensitivity of applied method.

The samples of selenium supplements were subjected to IC analysis once a month. The samples were stored at the following conditions:

– closed bottles at room temperature,
– closed bottles in refrigerator,
– opened bottles at room temperature,
– opened bottles in refrigerator.

It was stated that storage conditions of closed bottles have no effect on the quality of supplement – no changes was observed. The selenium ions did not undergo oxidation. The stability of SEL-BRCA1®, stored at room temperature and in the refrigerator in the closed bottles, can be confirmed for the period of time up to 45 months after the production date.

The small amounts of Se^{6+} ions was indicated in the samples of supplements stored in the opened bottles after 12 months of storage, irrespective of storage conditions (see Tables 2 and 3).

Thus, the stability of SEL-BRCA1®, stored in the open bottles can be confirmed for the period of time up to 12 months after the production date.

There were no bacteria or mold growth on plates, independently from used media (Soyabean Casein Digest Agar or Thioglycollate Agar) and incubation temperature (25°C or 37°C). During two years of studies no changes in bacteria number were observed.

Our results were in agreement with results obtained by commercial POLCARGO INTERNATIONAL SP Laboratory. After one year of storage, the pathogenic bacteria such as *Salmonella* spp. as well as *Escherichia coli* were not detected. Moreover, none of the samples under study contained coagulase-positive staphylococci, yeasts and molds. The 70% ethanol used as a selenium salt solvent is a preservative agent and enhances final supplements microbial stability.

It was shown that the percentage of microbiologically contaminated samples of selenium supplements was 0%. Patients taking SEL-BRCA1® and placebo can feel safe and there is no risk to their health.

Table 2. The concentration of selenium ions in the samples stored in the opened bottles at room temperature

	Se^{4+} [mg/dm^3]	Se^{6+} [mg/dm^3]
1	886.06	0.366
2	877.708	0.369
3	901.262	0.389

Table 3. The concentration of selenium ions in the samples stored in the opened bottles in the refrigerator

	Se^{4+} [mg/dm^3]	Se^{6+} [mg/dm^3]
1	897.33	0.375
2	879.03	0.358
3	884.341	0.363

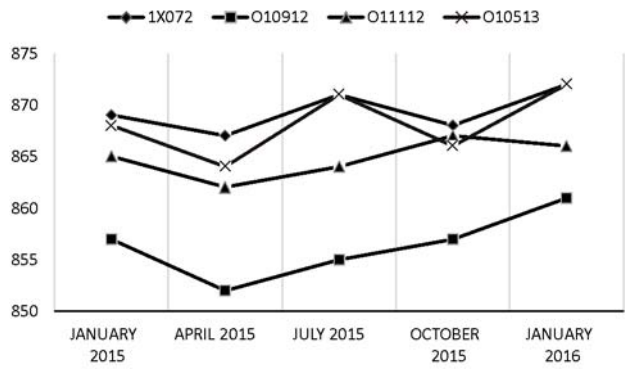

Figure 3. The concentration of SeO_4^{2-} ions in the supplement during storage time

CONCLUSIONS

The obtained results showed that:

– Concentration of SeO_3^{2-} ion in analyzed samples does not show any significant changes.

– Concentration of SeO_4^{2-} ion in analyzed samples (stored in the closed bottles) is below the limit of sensitivity of applied method.

– The stability of SEL-BRCA1® supplement, stored in the closed bottles, can be confirmed for the period of time up to 45 months after the production date.

– It is not recommended to store opened bottles for the period of time longer than 12 months.

– The fact that SEL-BRCA1® supplements show very high microbial stability underscores their usefulness as human medicines.

ACKNOWLEDGEMENTS

This work was supported by The National Center for Research and Development of Poland under project INNOMED/I/16/NCBR/2014.

LITERATURE CITED

1. Duntas, L.H. & Benvenga, S. (2001). Selenium: an element for life. *Endocrine* 48(3), 756–775. DOI: 10.1007/s12020-014-0477-6.

2. Brown, K.M. & Arthur, R. (2001). Selenium, selenoproteins and human health: a review. Public Health Nutr. 2001; 4(2b), 593–599. PMID:11683552.

3. Rayman, M.P. (2009). Selenoproteins and human health: insights from epidemiological data. *Biochim. Biophys. Acta* 1790(11), 1533–1540. DOI: 10.1016/j.bbagen.2009.03.014.

4. Rayman, M.P. (2012). Selenium and human health. Lancet 379, 1256–1268. DOI: 10.1016/S0140-6736(11)61452-9.

5. Hurst, R., Armah, C.N., Dainty, J.R., Hart, D.J., Teucher, B., Goldson, A.J., Broadley, M.R., Motley, A.K. & Fairweather-Tait, S.F. (2010). Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. *Am. J. Clin. Nutr.* 91, 923–31. DOI: 10.3945/ajcn.2009.28169.

6. Ning, Wang, Hor-Yue, Tan, Sha, Li, Yu, Xu, Wei, Guo & Yibin, Feng. (2017). Supplementation of Micronutrient Selenium in Metabolic Diseases: Its Role as an Antioxidant. Oxid. Med. Cell. Longev. Article ID 7478523, 13 pages. DOI: 10.1155/2017/7478523.

7. Postaire, E., Le Hoang, M.D., Anglade, P., Martinez, D., Brion, F., Navarro, J., Prognon, P. & Pradeau, D. (1989). Stability and behaviour of selenium in total parenteral nutrition solutions. *Int. J. Pharm*. 55(2–3), 99–103. DOI: 10.1016/0378-5173(89)90029-X.

8. Bagnicka, E., Kościuczuk, E.M., Jarczak, J., Jóźwik, A., Strzałkowska, N., Słoniewska, D. & Krzyżewski, J. (2017). The effect of inorganic and organic selenium added to diets on milk yield, milk chemical and mineral composition and the

blood serum metabolic profile of dairy cows. *Anim. Sci. Pap. Rep*. 35(1), 17–33.

9. Berzelius, J.J. (1817). Letter from Mr. Berzelius to Mr. Berthollet on two new metals. Ann. Chim. Phys. 187, 199–200.

10. Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E. & Hurst, R. (2011). Selenium in human health and disease. Antioxid. Redox Signal. 14(7), 1337–1383. DOI: 10.1089/ars.2010.3275.

11. Jablonska, E., Gromadzinska, J., Klos, A., Bertrandt, J., Skibniewska, K., Darago, A. & Wasowicz, W. (2013). Selenium, zinc and copper in the Polish diet. *J. Food Compost. Anal*. 31(2), 259–265. DOI: 10.1016/j.jfca.2013.05.016.

12. Jaworska-Bieniek, K., Lener, M., Muszyńska, M., Serrano-Fernández, P., Sukiennicki, G., Durda, K., Gromowski, T., Gupta, S., Kładny, J., Wiechowska-Kozłowska, A., Grodzki, T., Jaworowska, E., Lubiński, J., Górecka-Szyld, B., Wilk, G., Huzarski, T., Byrski, T., Cybulski, C., Gronwald, J., Dębniak, T., Ashuryk, O., Tołoczko-Grabarek, A., Morawski, A., Scott, R.J., Jakubowska, A. & Lubiński, J. (2015) Selenium and cancer. 377–390. Royal Society of Chemistry, In: Selenium: Chemistry, Analysis, Function and Effects (1st ed.)

13. Commission of the European Communities, Nutrient and energy intakes for the European Community. (1993). Reports of the Scientific Committee for Food, thirty first series, Office for Official Publications of the European Communities, Luxembourg.

14. Farmakopea Polska VI. Polskie Towarzystwo Farmaceutyczne (in Polish) 2002.