

# ATOMIC FORMS OF SELENIUM IN SELECTED MINERAL AND WELL WATERS IN POLAND

## FORMY SELENU W MINERALNYCH I PITNYCH WODACH W POLSCE

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**Słowa kluczowe:** *selen, analiza, specjacja, wody mineralne, wody studzienne*

### ABSTRACT

The study aimed at application of a high performance liquid chromatography (HPLC) with UV detector for selenium speciation in samples of mineral and well waters in Poland. Basic solutions 1000 µg/l  $\text{SeO}_3^{2-}$  and  $\text{SeO}_4^{2-}$  were prepared by dissolving 2.190 g of reagent  $\text{Na}_2\text{SeO}_3$  and 2.393 g  $\text{Na}_2\text{SeO}_4$  in 1000 ml of deionised water; respectively. Recent standards  $\text{SeO}_3^{2-}$  and  $\text{SeO}_4^{2-}$  (single or mixed) were prepared daily. Chromatographic separations were obtained on a column with reversed phase of C18, 250\*4 mm, 5 µm ODS. As mobile phase mixture of 5 mmol/l phosphate tetrabutylammonium, 50/50 water/methanol at pH 3.4 was used. Flow rate of mobile phase was 1 ml/min and detector was set on  $\lambda=210$  nm. Injection volumes were 5 µl. The average concentration of Se(IV) was 4.10 µg/l and Se(VI) - 4.27 µg/l and Se(II) - 0.54 µg/l although the differences between water samples originating from various sources were relatively high. The developed method can be therefore recommended for determination of Se in samples of mineral water.

### STRESZCZENIE

Celem pracy było wykorzystanie metody HPLC do badania specjacji selenu w mineralnych i pitnych wodach. Roztwory podstawowe  $\text{Na}_2\text{SeO}_3$  i  $\text{Na}_2\text{SeO}_4$  o stężeniu 1000 µg/l przygotowano przez rozpuszczenie 2,190 g  $\text{Na}_2\text{SeO}_3$  i 2,393 g  $\text{Na}_2\text{SeO}_4$  w 1000 ml wody dejonizowanej. Świeże standardy  $\text{SeO}_3^{2-}$  i  $\text{SeO}_4^{2-}$  (pojedyncze lub mieszane) były przygotowane codziennie, z roztworów podstawowych. Rozdziały chromatograficzne uzyskano na kolumnie z fazą odwróconą C18, 250\*4 mm, 5 µm ODS. Fazą ruchomą była mieszanina 5 mmol/l fosforanu tetrabutylamoniowego, 50/50 woda/metanol o pH 3,4. Szybkość przepływu fazy ruchomej wynosiła 1ml/min. W badaniach stosowano detekcję przy długości fali  $\lambda=210$  nm. Objętość badanych próbek wynosiła 5 µl. Wszystkie warunki pomiarów uprzednio zoptymalizowano. Średnia zawartość Se(IV) w badanych wodach wynosiła 4,10 µg/l, a Se(VI) 4,27 µg/l i Se(II) 0,54 µg/l, chociaż obserwowano znaczne różnice w zależności od pochodzenia próbki. Opracowana metoda RP-HPLC-UV może być wykorzystana do badania specjacji selenu w próbkach wody mineralnej.

### INTRODUCTION

The speciation analysis of ions and non-dissociated species is often applied in the investigation of sludge, biological materials, food products and other environmental samples [6, 14].

With an increasing knowledge of the biological functions of selenium, the growing interest in the analytical methods for the determination of trace amounts of this element including its speciation in environmental and biological samples is obvious [11, 25]. For separation of mixtures various types of chromatography are most frequently used, while different spectral methods and

especially atomic absorption spectrometry are used for quantification [16, 20, 25]. The methods most often used for the determination of organic and inorganic selenium are shown in the Table 1 [5, 12, 22].

Combined techniques which include analyte enrichment and separation, allow to achieve the delectability threshold at  $\leq 1$  ng/dm<sup>3</sup>. The speciation analysis is a modern and still developing chemical analytical approach which provides qualitative data and quantitative indices related to various species of elements [13]. The bioactivity and toxicity of selenium depends not only on its total concentration but also on chemical forms in which it occurs. The inorganic selenium is absorbed

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quickly by an organism, however it is also quickly excreted with urine, while about 75% of selenomethionine remains. Selenates (IV) are more toxic for animals than selenates (VI) [23]. The selenates (VI) belong to the best assimilated selenium species by plants [23].

The studies on selenium speciation most often involve samples of body liquids such as urine, blood and serum [26, 27]. At present, speciation of this element is also examined in soils and in plants. The methods of selenium speciation are used most frequently for surface waters. Current selenium speciation analytical techniques allow for the determination of various species including :

- volatile alkyl derivatives of selenium (CH<sub>3</sub>)<sub>2</sub>Se and (CH<sub>3</sub>)<sub>2</sub>Se<sub>2</sub>,
- non-volatile selenium compounds including inorganic selenium oxoanions, Se(IV) and Se(VI), as well as selenoamino acids, e.g. selenomethionine or selenocysteine [24].

These selenoaminoacids were determined in yeasts, urine and the certified reference materials. *Potin-Gautier* et al. [21] in white clover found 2 µg/g SeCys and 0,60 µg/g SeMet, and in the certified reference material 1,5 µg/g SeCys and 4,2 µg/g SeMet. *Gilon, Potin-Gautier and Astruc* [10] reported 343 – 365 mg/kg SeCys and 436 – 457 mg/kg SeMet in yeasts enriched with selenium. *Michalke and Schramel* [16] in human milk found 5,2 µg/dm<sup>3</sup> SeCys and 1 µg/dm<sup>3</sup> SeMet. *Pyrzyńska* [28] established that speciation analysis of non – volatile compounds of selenium occurring in natural waters involved determination of the content of selenium in three samples following their preparation: 1. In a primary sample the content of Se(IV) was determined with oxidation methods suitable for this stage of analysis. 2. Following the samples mineralization and oxidation of Se to organic forms (IV), the sum of Se(IV) + Se(II) was determined. 3. In the mineralised sample Se(VI) was reduced to Se(IV) with an aid of

hot concentrated HCl to determine the content of total selenium as the sum Se(IV), Se(VI) and Se(II)]. The ratios between Se(VI) and Se(II) were defined by a difference between individual samples. High performance liquid chromatography (HPLC) was used to investigate selenium species. The technique of hydrides generation combined with the AAS method can be applied for the final determination of selenium in biological samples such as foodstuffs or sewage since it enables to eliminate the influence of the matrix [1, 6]. Unlike many investigations on total content of selenium, little information has been published on inorganic speciation and organic forms of this element. Studies on speciation of selenium in Poland have up to now been very scarce. The articles by *Świetlik* [30], *Hulanicki* [13], *Niedzielski* [17-19] and *Pyrzyńska* [22-28] contain mainly theoretical considerations. *Dejneka* [2] carried out speciation of selenium in cereals, herbs and nutrition products for babies, showing insignificant content of Se(IV) and Se(VI). *Gawłowska-Kamocka's* carried out speciation of inorganic forms of selenium in confectionery products [9], Se(IV) and Se (IV) in mineral waters [7], inorganic forms of selenium in waters and other beverages [8].

The aim of this work was to implement reversed phase high performance liquid chromatography (RP-HPLC) with UV detection to study speciation of selenium in selected samples of mineral and well waters.

## MATERIAL AND METHODS

### Material

The experimental material consisted of 11 samples of different mineral water produced in Poland and distributed in 1.5 l plastic bottles in retail trade in the city of Łódź. This were following sparkling and still mineral waters: Muszynianka, Nałęczowianka, Oda, Multi Vita, Mazowszanka, Ostromecko citron, Zdroje Grodziska,

Table 1. The analytical techniques used in the analysis of selenium compounds  
Metody analityczne stosowane w specjacji związków selenu

Species	Matrix	Analytical method	Detection limit
Total Se	groundwater	HG-AAS	2 ng l <sup>-1</sup>
Total Se	sediment	HG-ICP-AES	0.40 µg l <sup>-1</sup>
Total Se	sediment	fluorimetry	0.28 µg l <sup>-1</sup>
Total Se	urine	HG-AAS	20 ng l <sup>-1</sup>
Se(IV), Se(VI)	coal ashes	IC-CD	50 µg l <sup>-1</sup>
Total Se	urine	GF-AAS	20 µg l <sup>-1</sup>
Se(IV)	shampoo	spectrophotometry	1 µg l <sup>-1</sup>
Total Se	human hair	polarography	-
Total Se	soil	CSV	0.75 µg l <sup>-1</sup>
Total Se	plants	HG-ICP-MS	1.3 ng l <sup>-1</sup>
Se(IV), Se(VI)	soil	IC-CD	30 µg l <sup>-1</sup>
DMSE	soil	GC-AAS	5 pg
Total Se	biological samples	HG-AAS	0.02 µg l <sup>-1</sup>
Diphenyl selenide	drain water	MEKC	30 pg l <sup>-1</sup>

Abbreviations: CD - conductometric detection, CSV- cathodic stripping voltametry, MEKC- micellar electrokinetic chromatography.

Krakowianka, Fructom and Galicjanka. Experiments covered also natural water samples taken from water intakes in Łódź.

Apparatus, reagents and solutions, samples processing prior to analysis, retention times of selenium compounds were described earlier in the articles by *Gawłowska* and *Masłowska* [7, 8]. In all tested water samples selenium species were determined by the high efficiency liquid chromatography (RP-HPLC) using a Dionex-Sofran GmbH instrument equipped with a DG-1310 degasser, p580A HPG pump, Gina 50T autosampler, STH 585 oven, UV/VIS UVD 170s detector with software Chromeleon version 6.00.

Reagents and solutions:

- Distilled-deionized water with conductivity 0,03  $\mu\text{S}$ .
- Methanol for HPLC, of analytical grade, POCh-Gliwice.
- 5 mmol/l tetrabutylammonium phosphate of analytical grade, Fluka.
- A stock solution of sodium selenate (IV)  $\text{Na}_2\text{SeO}_3$  (A) was prepared from a solid reagent of analytical grade, Fluka by dissolving 2.1900 g  $\pm 0,0001$  g of  $\text{Na}_2\text{SeO}_3$  in distilled-deionised water in a 1000 ml measuring flask.
- A stock solution (B) of sodium selenate (VI)  $\text{Na}_2\text{SeO}_4$  was prepared from a solid reagent of analytical grade, Fluka by dissolving of 2.3930 g  $\pm 0,0001$  g  $\text{Na}_2\text{SeO}_4$  in distilled-deionised water in a 1000 ml measuring flask.

Standard solutions of  $\text{SeO}_3^{2-}$  and  $\text{SeO}_4^{2-}$  (single or mixed) were prepared daily from stock solutions A and B.

## Method

In preliminary experiments good chromatographic separations were achieved in a reversed-phase C18 column, 250 $\times$ 4.0; 5  $\mu\text{m}$  ODS. The mixture of 5 mmol/l of tetrabutylammonium phosphate, 50/50 water/methanol at pH 3.4 was chosen as a suitable mobile phase with the flow rate 1 ml/min. The detection of analytes was carried out at  $\lambda=210$  nm. Sample solutions were filtered on Filtrak Sorte 390 filters of  $\phi$  125 mm (Ref. – No. 3.103.125) before being injected into the column. The volume of solutions of the tested samples was constant and equal to 5  $\mu\text{l}$ .

## RESULTS AND DISCUSSION

The results of the determinations of three different forms of selenium as well as total selenium concentration in waters can be found in Table 2. Se(II) was not found in 6 samples and its concentration in the remaining samples was found to be lowest, ranging between 0.10 and 9.74  $\mu\text{g/l}$ . Concentration of Se(IV) ranged from 1.42 to 16.42  $\mu\text{g/l}$ . The data in the Table 2 show that the waters with higher concentration of Se(II) were also characterised by highest Se(VI) content ranged from 0.94 to 20.08  $\mu\text{g/l}$ . The highest total selenium concentration was found in the water from Barycz well and the deep well in the brown coal mine in Bełchatów. The lowest content of Se(II) ( $0.10 \pm 0.008$   $\mu\text{g/l}$ ) was found in mineral water "Oda". The highest content of Se(IV) was reported in well water Barycz. The concentration of selenium in the examined samples

Table 2. Se(IV), Se(VI), Se(II) and total selenium content in mineral and well waters determined with RP-HPLC-UV method.

Zawartości Se(IV), Se(VI), Se(II) i selenu całkowitego w mineralnych i pitnych wodach oznaczone metodą RP-HPLC-UV.

No	Kind of water	Se(IV) $\pm$ SD [ $\mu\text{g/l}$ ]	Se(VI) $\pm$ SD [ $\mu\text{g/l}$ ]	Se(II) $\pm$ SD [ $\mu\text{g/l}$ ]	Total (Se) $\pm$ SD [ $\mu\text{g/l}$ ]
1	Oda *	6.84 $\pm$ 0.40	7.82 $\pm$ 0.30	0.10 $\pm$ 0.08	14.70 $\pm$ 0.11
2	Oda	7.84 $\pm$ 0.35	8.02 $\pm$ 0.95	0.16 $\pm$ 0.05	15.10 $\pm$ 0.63
3	Muszynianka	4.68 $\pm$ 0.20	4.90 $\pm$ 0.12	0.18 $\pm$ 0.09	9.76 $\pm$ 0.01
4	Nałęczowianka	4.69 $\pm$ 0.30	4.80 $\pm$ 0.15	1.78 $\pm$ 0.58	13.60 $\pm$ 0.15
5	Multi Vita	3.08 $\pm$ 0.16	3.99 $\pm$ 0.08	0.45 $\pm$ 0.20	7.54 $\pm$ 0.10
6	Mazowszanka	3.80 $\pm$ 0.18	4.09 $\pm$ 0.15	-	7.82 $\pm$ 0.30
7	Galicjanka *	2.69 $\pm$ 0.25	0.94 $\pm$ 0.55	1.00 $\pm$ 0.67	3.10 $\pm$ 0.75
8	Zdroje Grodziska*	2.14 $\pm$ 0.25	1.54 $\pm$ 0.25	2.10 $\pm$ 0.68	12.45 $\pm$ 0.35
9	Krakowianka *	5.42 $\pm$ 0.21	5.72 $\pm$ 0.14	0.31 $\pm$ 0.18	11.46 $\pm$ 0.30
10	Ostromecko citron*	1.42 $\pm$ 0.10	2.23 $\pm$ 0.16	-	3.65 $\pm$ 0.17
11	Fructom *	2.50 $\pm$ 0.11	3.00 $\pm$ 0.30	-	5.50 $\pm$ 0.15
12	Source 'Malinka' **	9.45 $\pm$ 0.57	12.34 $\pm$ 1.71	-	21.79 $\pm$ 1.08
13	Deep-well 5N bis ***	12.14 $\pm$ 1.15	15.56 $\pm$ 1.90	9.74 $\pm$ 2.16	38.09 $\pm$ 1.34
14	Deep-well 6N bis ***	15.67 $\pm$ 1.25	19.84 $\pm$ 3.78	4.24 $\pm$ 2.10	39.80 $\pm$ 2.35
15	Well water – Zgierz	7.25 $\pm$ 0.89	7.54 $\pm$ 0.20	0.77 $\pm$ 0.98	15.56 $\pm$ 3.36
16	Well water – Łęczyca	8.50 $\pm$ 1.12	15.72 $\pm$ 1.23	-	24.22 $\pm$ 1.20
17	Well- Barycz ****	16.42 $\pm$ 1.10	20.08 $\pm$ 3.30	3.90 $\pm$ 1.45	40.43 $\pm$ 1.80
18	Well water – Łódź	7.56 $\pm$ 0.98	10.85 $\pm$ 1.12	-	18.41 $\pm$ 1.00

\* - sparkling water, \*\* - Zgierz, \*\*\* - the brown coal mine in Bełchatów, \*\*\*\*- province Świętokrzyskie

decreases in the following order: Barycz (well) < 6N bis (deep-well) < 5N bis (deep-well) < Łęczyca (well) < "Malinka" (source) < Łódź (well) < Zgierz (well) < "Oda" < "Nałęczowianka" < "Zdroje Grodziska" < "Krakowianka" < "Muszynianka" < "Mazowszanka" < "Multi Vita" < "Fructom" < "Ostromecko citron" < "Galicjanka". Earlier, *Sikorowska* [29] and *Masłowska* [3, 7, 15] obtained similar results of selenium concentrations in waters. Much higher levels of selenium were found in all tested water samples as compared to the results given for Finnish waters by *Varo et al.* [31], which is related to a fact that selenium content in water samples and food in Finland is relatively low. In this study much higher selenium concentrations were found in all tested water samples as compared to the results given by *Masłowska* and *Gawłowska* [8] in the year 2002, which probably results from the use different mobile phase and mineralization technique as also from different producers of the examined waters. The average concentrations of Se(IV) were 4.10 µg/l and Se(VI) – 4.27 µg/l and Se(II) – 0.54 µg/l. These levels do not exceed the WHO [4] recommended amount of 0.010 mg/l (10 µg/l) in any of the analyzed samples. These results indicate that the concentration of selenium in mineral waters is low and might not cover selenium daily demand. However, the recommendation on enrichment the diet with selenium should be preceded by a study of total dietary selenium intake.

## CONCLUSIONS

The results of this study proved that the RP-HPLC-UV technique provides a useful tool for distinguishing and simultaneous determination of three ion forms of Se(II), Se(IV) and Se(VI) in samples of waters. The developed method can be therefore recommended for determination of selenium in samples of mineral water and food products.

In summary, it can be assumed that the level of selenium in natural waters in Poland is very low and might not cover the demand for this element. Therefore, the deficiency of this microelement in the diet might be reduced by increasing the consumption of selenium enriched products. However, the studies of total selenium dietary intake from all sources should be carefully drafted and evaluated before diet supplementation with this element could be recommended.

## REFERENCES

1. *Astruc M.*: Recent advances in speciation methods: arsenic and selenium. *J. Trace Elem. Exper. Med.* 1998, 11, 333.
2. *Dejneka W.*: Analiza specjacyjna nieorganicznych form selenu w środkach spożywczych. *Bromat. Chem. Toksykol.* 2002, 35, 35-40.
3. *Duda J., Masłowska J.*: Polarograficzna metoda oznaczania śladowych ilości selenu w wodach pitnych i napojach. *Bromat. Chem. Toksykol.* 1985, 1, 11-16.
4. Environmental Protection Agency Recommendation of the National Academy of Sciences, Federal Register 1997, 42, 3573.
5. *Florence M.T.*: The speciation of trace element in water. *Talanta* 1982, 29, 345-364.
6. *Gawłowska A., Masłowska J.*: Specjacja jonów pierwiastków chemicznych w próbkach naturalnych. *Zesz. Nauk. P. Ł. Chemia Spoż. i Biotech.* 2000, nr 832, z.62, 13-29.
7. *Gawłowska A., Masłowska J.*: Wykorzystanie metody HPLC do oznaczania Se(IV) i (VI) w wodach mineralnych. *Żywność. Nauka. Technologia. Jakość* 2001, 3(28), 86-94.
8. *Gawłowska A., Masłowska J.*: Analiza specjacyjna nieorganicznych form selenu w wodach i innych napojach. *Bromat. Chem. Toksykol.* 2002, 35, 361-368.
9. *Gawłowska - Kamocka A.*: Speciation analysis of inorganic forms of selenium in confectionery products. *J. Elementol.* 2007, 12(2), 105-110.
10. *Gilon M., Potin-Gautier M., Astruc M.*: Optimization of the determination of inorganic and organic selenium species using high-performance liquid chromatography-electrothermal atomic absorption spectrometry. *J. Chromatogr. A.* 1996, 750, 327-334.
11. *Godlewska B., Hulanicki A.*: Specific problem of speciation study in biological materials. *Chem. Anal.* 1993, 3, 267-277.
12. *Guerin T., Astruc A., Astruc M.*: Speciation of arsenic and selenium compounds by HPLC hyphenated to specific detectors: a review of the main separation techniques. *Talanta* 1999, 50, 1-24.
13. *Hulanicki A.*: Analiza specjacyjna próbek biologicznych. *Wiad. Chem.* 1997, 3/4 189-203.
14. *Łukasiak J.*: Analiza specjacyjna w badaniu żywności. *Bromat. Chem. Toksykol.* 1995, 28, 197-208.
15. *Masłowska J.*: Woda mineralna w Piaskach z ujęć odwodnień KWB „Bełchatów”. *Węgiel Brunatny* 1993, 67, 9-11.
16. *Michalke B., Schramel P.*: Selenium speciation in human milk. *Z. Ernährungswiss.* 1997, 1, 68-69.
17. *Niedzielski P., Siepak J., Kowalczyk Z.*: Speciation analysis of arsenic, antimony and selenium in waters of the Lednickie Lake. *Archives Environ. Protection*, 2000, 26(1), 73-82.
18. *Niedzielski P., Siepak J.*: Speciation analysis of arsenic, antimony and selenium in city area surface waters. *Chem. Environ. Res.* 2001, 10, 117-219.
19. *Niedzielski P., Siepak J., Siepak M.*: Analiza specjacyjna arsenu, antymonu i selenu w wodach mineralnych i wodociągowych. *Przyroda i Człowiek.* 2000, 10, 183-191.
20. *Potin-Gautier M.*: Interet de la speciation: l'exemple du selenium. *Analysis* 1997, 25, M22-M27.

21. *Potin-Gautier M., Gilon N., Astruc M., de Gregori I., Pinochet H.*: Comparison of selenium extraction procedures for its speciation in biological materials. *Intern. J. Environ. Anal. Chem.* 1997, 67, 15-25.
22. *Pyrzyńska K.*: Recent development in speciation analysis of selenium. *Chem. Anal.* 1995, 40, 677-686.
23. *Pyrzyńska K.*: Speciation of selenium compounds. *Anal. Sci.* 1998, 14, 479-483.
24. *Pyrzyńska K.*: Związki selenu w środowisku naturalnym. *Wiad. Chem.* 2000, 1/2, 139-150.
25. *Pyrzyńska K., Bulska E., Gucer S., Hulanicki A.*: Aluminium speciation in natural waters. *Chem. Anal.* 1993, 11-14.
26. *Pyrzyńska K.*: Speciation analysis of some organic selenium compounds. A review. *Analyst*, 1996, 121(8), 77 R – 83 R.
27. *Pyrzyńska K.*: Speciation analysis of selenium. IV International Symposium, Warsaw 1998.
28. *Pyrzyńska K.*: Specjacja selenu w wodach naturalnych. Red. *J. Siepak*, Analiza specjacyjna metali. Wyd. UAM, Poznań 1998.
29. *Sikorowska C.*: Selen w wodach wodociągowych w Polsce. *Roczn. PZH* 1965, 16, 11-15.
30. *Świetlik R.*: Znaczenie pojęcia specjacja w chemii środowiska. *Wiad. Chem.* 1998, 7/8, 587-595.
31. *Varo P., Nuurtarmo H., Saari P., Koivostoinen P.*: Selenium content of cereal grains, flour and bread produced in Finland. *Acta Agr. Scand. Suppl.* 1980, 22, 37.

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