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TECHNICAL NOTE

TOXICOLOGY

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Application of Three Spectrometric Methods to Total Selenium Determination in Postmortem Material in a Case of Acute Selenium Compound Poisoning

ABSTRACT: Three spectrometric methods, that is, spectrofluorimetry (SF), atomic absorption spectrometry with electrothermal atomization (ET-AAS), and atomic fluorescence spectrometry with hydride generation (HG-AFS) were used for the determination of total selenium in biological samples taken from postmortem material in a case of acute selenium compound poisoning. The precision of the SF, ET-AAS, and HG-AFS methods (RSD, $n = 10$) was found to be in the ranges of 10.0–15.0, 3.0–6.0 and 1.0–1.5%, respectively, and the detection limit was 10.0, 4.0, and 0.1 μg/L of Se, respectively. In the case of HG-AFS, the analytical procedure takes less time and is less laborious than the other methods considered. The obtained results show the usefulness of the HG-AFS method as a supplementary analytical tool to the SF and ET-AAS methods with respect to the determination of selenium as well as the possibility of using this method as a primary one in forensic toxicology practice.

KEYWORDS: forensic science, fatal poisoning, postmortem material, total selenium, spectrofluorimetry, electrothermal atomization atomic absorption spectrometry, atomic fluorescence spectrometry with hydride generation

Selenium is an essential trace element at low concentrations in many species, including humans, but toxic at high concentrations (with an approximately three to fourfold difference in blood/serum concentrations in regards to toxicity; [1–3]). It is a critical nutrient for animals, and for human health in the range 40–100 μg per day, but can be toxic above this value (depending on age; [1]). The essential role of selenium is due to its presence in the active sites of some enzymes (e.g., glutathione peroxidases, dehydrogenases, iodothyronine-5′-deiodinase), selenoproteins (e.g., selenoprotein P in serum; [4,5]), the catalytic effects of selenium compounds on reactions of intermediate metabolism, and inhibition of the toxic effect of heavy metals, such as Ag, Hg, and Cd, by formation of stable and less toxic compounds (2,6). Moreover, selenium is one of the constituents of the detoxification system in the human and animal organism (including detoxification of organic compounds released during infections, traumas, and stresses), and is involved in maintaining homeostasis (1). Acute selenium toxicity has been observed in experimental animals (7). Reports of acute selenium poisoning in humans are rare (5,8–10); only about 20 cases were reported until 2008 (11).

Consequently, it is important to determine selenium in internal tissues and body fluids to investigate the effect of selenium on human health or to evaluate a poisoning. The determination of selenium in biological and environmental samples requires sensitive and accurate analytical techniques. Care should also be taken to avoid sources of systematic error including loss of volatile selenium compounds in all the steps from sampling to the final measurement (2,12). Determination of selenium in body fluids and tissues is usually carried out by molecular fluorescence spectrometry, that is, spectrofluorimetry (SF; [13–15]), atomic absorption spectrometry using either hydride generation or direct electrothermal atomization (HG-AAS or ET-AAS [8,15–18]), atomic fluorescence spectrometry with hydride generation technique (HG-AFS; [19–24]), gas chromatography-mass spectrometry (GC-MS [10]), neutron activation analysis (NAA; [25]), and other methods (18). The modern method of inductively coupled plasma-mass spectrometry (ICP-MS; [26–28]) has some advantages over rival techniques, including low detection limits and speed of analysis; however, not all determinations are straightforward, in particular, the determination of selenium in serum—the sensitivity with conventional ICP-MS is generally poor because only 30% ionization is achieved with an argon plasma and that there are relatively high spectroscopic interferences caused by the formation of argon polyatomic species (26). Moreover, NAA and ICP-MS methods are not readily accessible by many laboratories (at least in Poland) because their running costs are very high. Determination of selenium in body fluids by inductively coupled plasma-optical emission spectrometry (ICP-OES) in the range of 0.1–100 mg/L is burdened with spectral (most probably from CO molecules) and nonspectral (from C, S, P and Br) matrix interferences (29,30). ET-AAS is the most common method for analysis of trace elements in biological fluids and solid samples (8,15,16). Generally, this technique has been used in routine casework concerning fatal metal poisoning, whereas the SF method serves as a complementary method for selenium determination in different materials.

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The aim of this work was to compare three spectrometric methods: SF, ET-AAS, and HG-AFS for the determination of total selenium in biological materials for forensic purposes. Both the SF and ET-AAS methods are routinely used in the Institute of Forensic Research in Krakow in casework concerning fatal metal poisoning and the obtained results have been submitted in court. The HG-AFS procedures have been developed in the Laboratory for Forensic Chemistry at the Jagiellonian University for determination of total selenium in human blood and hair (19,20). In this work, all of these methods were applied to analysis of postmortem material collected in a case of acute poisoning.

Materials and Methods

Reagents and Samples

Standard stock solutions containing 1000 mg/L Se were prepared from Titrisol standards (Merck, Darmstadt, Germany). For digestion procedures: concentrated HNO_3, H_2SO_4, and $HClO_4$ (Merck) were used. For quantification, the following were used: concentrated HCl (POCh, Gliwice, Poland); 2% (m/v) $NaBH_4$ (Merck); 0.5% (m/v) $NaBH_4$; 2,3-diaaminonaphthalene (DAN; [Sigma-Aldrich, St. Louis, MO]) solution (100 mL 0.1 M HCl + 500 mg $NH_2OH.HCl$ + 100 mg DAN) prepared in semi-darkened room or in room in only yellow light, fresh for each set of determinations, purified by double extraction with 10 mL cyclohexane, Na-EDTA, NaF (POCh) in solution (74.5 g Na-EDTA and 6.7 g NaF/L); and palladium modifier (Merck; 10 g $Pd(NO_3)_2$/L in 15% HNO_3), diluted 10 times before analysis.

All reagents were analytical grade. Doubly deionized water (<1.0 μS/cm) from Barnstead (Dubuque, IA) was used throughout.

Standard reference material (Bovine Liver SRM 1577b; National Institute of Standards and Technology, Gaithersburg, MD) was examined.

Case Study and Postmortem Examination

A 22-year-old student of biology, who was carrying out experiments for her Master's thesis, ingested about 20 mL of sodium tetraoxoselenate(VI) with suicidal intent. After admission to hospital (with vomiting, severe diarrhea, and abdominal pains), she developed cardiovascular failure, and a strange smell emanated from her whole body. Postmortem examination revealed cerebral edema, focal fibromatosis of the heart, and massive congestion with focal hemorrhage in the lungs. In the stomach, there was a focal superficial necrosis of the gastric mucosa and granulocytic infiltration in the submucosa.

Autopsy material (sections of internal organs: stomach, liver, kidney, lung, and blood sample) was subjected to chemical-toxicological examination for selenium by use of the three methods mentioned above. Two wet digestion methods described below were used to decompose the organic matrix of all samples.

Digestion Procedures

Method A—wet digestion in a Bethge apparatus (LABART, Gdansk, Poland): 1 mL of blood sample or 1 g of homogenized tissue, or 0.5 g of lyophilized certified reference material was mineralized in a Bethge apparatus, using 9 mL of concentrated nitric acid and 3 mL of concentrated perchloric acid followed by oxidation with 2 × 1 mL 30% hydrogen peroxide. The final volume of the solution was 30 mL.

Method B—microwave digestion in the system MARS X (CEM Corp., Matthews, NC): 0.5 mL of blood sample or 0.5 g of a sample of a homogenized tissue or the reference material was placed in a high-pressure teflon vessel, 7 mL of concentrated HNO_3 was added and then the vessel was closed and transferred into a microwave oven for digestion. The program of mineralization is described in detail in Table 1. After digestion, the vessel was cooled to a temperature of 25°C. Then it was opened and the gas above the sample was removed by a stream of nitrogen. To avoid the strong negative influence of nitrogen oxides on the analytical results (20,31), nitrogen oxides were removed from the mineralized sample using a stream of inert gas (nitrogen) for 10 min. Then the sample was transferred into a 25 mL volumetric flask, and 12.5 mL of 6 M hydrochloric acid (as a pre-reduction reagent) was added and the solution was diluted to the mark with water.

SF Method

Selenium(VI) in the digestion solution, 1–10 mL (method A) was reduced with 10 mL of 6 M hydrochloric acid at 100°C (1 h) to selenium(IV) and then quantified by the SF method using procedures modified by Tamari et al. (13) and Petterson et al. (14) procedures. Complexes of selenium with 5 mL DAN (piazselenols) were extracted into cyclohexane (2 × 10 mL) at pH 1.8 in the presence of 2 mL EDTA-NaF solution, shaken for 2 min, and after 5 min separated. The detection limit was 10 ng of selenium. A fluorescence spectrometer F-2000 from (Hitachi Ltd, Tokyo, Japan), equipped with xenon lamp 1.0-cm (pathlength) quartz cuvettes, was used for measurements. The excitation wavelength was 366 nm; the emission (fluorescence) was measured at 554 nm.

ET-AAS Method

After digestion (method A), the sample was transferred into a 15 mL volumetric flask and diluted to the mark with water. Then a 10 μL aliquot of a diluted solution of palladium modifier was introduced into a graphite tube followed by 20 μL of digested sample. Measurements were carried out with the use of a Pye Unicam 9100X atomic absorption spectrometer (Philips, Cambridge, Great Britain), with deuterium background correction. The signals were obtained using an ashing temperature of 1100°C and an atomization temperature of 2400°C. The standard addition method was used for quantification.

HG-AFS Method

The sample solution after digestion (method B) was introduced into the flow hydride generation system and merged with a solution containing 2% (m/v) sodium tetrahydroborate and 0.5% (m/v) sodium hydroxide. Hydrochloric acid at a concentration of 3 M played the role of a carrier solution. A double-channel atomic fluorescence spectrometer AFS-230 (Beijing Haiguang Instrument Co., Beijing, China), equipped with a 130-positional autosampler and a flow hydride-generation system with an intermittent flow method was used for the measurement of analytical signals. The light source was a cathode lamp (Se-HCL) with an operating current

TABLE 1—*Parameters of the optimized microwave digestion procedure.*

Stage	Power (%)	Temperature (°C)	Pressure (Psi)	Ramp Time (Min)	Hold Time (Min)
I	90	180	340	4.00	4.00
II	90	200	350	4.00	4.00
III	95	220	360	4.00	4.00

TABLE 2—*Comparison of analytical parameters of the three methods considered for standard solutions.*

Parameter	Analytical Method		
	SF	ET-AAS	HG-AFS
Calibration	Set of standards method	Standard addition method	Set of standards method
Linearity range, μg/L	10.0–500.0	0.4–80.0	0.1–100.0
Detection limit, μg/L*	10.0	0.4	0.1
RSD, % (n = 10)	15.0 (44 μg Se/L)	6.2 (40 μg Se/L)	1.0 (10 μg Se/L)
	10.4 (90 μg Se/L)	3.0 (80 μg Se/L)	1.5 (100 μg Se/L)
Time of preparation and measurement (8–10 samples)	16 h	8 h	2 h

*The detection limit was calculated according to the formula: LOD = 3 SD_B/a, where SD_B is the standard deviation of the analytical signal for 10 blank samples and a is the slope of the calibration curve (36).

ET-AAS, electrothermal atomization atomic absorption spectrometry; HG-AFS, atomic fluorescence spectrometry with hydride generation; RSD, relative standard deviation; SF, spectrofluorimetry.

%RSD = (standard deviation/mean) × 100.

(pulsed value) of 100 mA. Argon was used as the shield and the carrier gas with a flow of 800 and 500 mL/min, respectively. The atomization process occurred in an Ar-H_2 flame at a temperature of 200°C. Conventional methods were used for calibration.

Results and Discussion

The procedures for the determination of selenium by means of the three considered methods were validated using both standards and real samples. The most important defined analytical parameters are presented in Table 2. The SF method—very popular in routine work—is selective for selenium and provides an opportunity to determine this element in a wide concentration range. However, it is time-consuming and provides analytical results that are poor in terms of precision. As can be seen, selenium can be determined in better analytical conditions by means of ET-AAS, which is characterized by good sensitivity and precision. The problem is the matrix effect occurring in this method as spectral (caused by phosphorus species or atomic iron) and volatilization (selenium may be lost in low temperature) interferences. For this reason, the standard addition method has to be applied for quantitation with use of different modifiers, which makes this technique more time-consuming and expensive. HG-AFS turned out to be the method of choice, exhibiting the best sensitivity and precision as well as sufficient linearity. Interferences, which could be caused by nitrogen oxides, can easily be avoided at the digestion step; hence, it is possible to calibrate using a set of standards. In addition, as the microwave digestion process is relatively simple and fast, the complete analytical procedure takes the shortest time. Another advantage of HG-AFS is the very low detection limit (due to low background noise), although for both SF and ET-AAS, the detection limit was found to be low enough to determine selenium contained in the biological samples examined.

Selenium was determined in reference material 1577b Bovine Liver (National Institute of Standards and Technology) and in postmortem material taken from the autopsy in the mentioned case of poisoning of the young woman (22-years-old). The analytical results obtained are shown in Table 3.

The examinations performed proved that all three considered methods are able to provide very good results in terms of accuracy (compared with the certified value of Se in the reference material). The reason for the differences between the selenium content found by the three methods in individual tissues might lie in difficulties in making the samples perfectly homogeneous before the digestion process (in blood samples, which were free of such difficulties, selenium was determined to be at the same concentration). It is also seen again that the results obtained by HG-AFS are, as a rule, much more repeatable than those obtained by the SF and ET-AAS methods.

TABLE 3—*Results of selenium determination (*n = *3) in reference material (with certified value of 0.73 ± 0.06 μg/g) and in postmortem tissues, obtained by the three considered spectrometric methods.*

Material	Selenium Content (μg/g)		
	SF	ET-AAS	HG-AFS
1577b bovine liver	0.75 ± 0.06	0.74 ± 0.04	0.72 ± 0.03
Stomach	4.67 ± 0.68	4.65 ± 0.26	4.50 ± 0.02
Liver	4.35 ± 0.87	4.20 ± 0.46	3.85 ± 0.08
Kidney	3.40 ± 0.59	3.35 ± 0.10	2.52 ± 0.02
Lung	1.60 ± 0.21	1.80 ± 0.20	1.80 ± 0.01
Blood*	1410.0 ± 70.0	1410.0 ± 50.0	1410.0 ± 10.0

*The values are given in μg/L.

ET-AAS, electrothermal atomization atomic absorption spectrometry; HG-AFS, atomic fluorescence spectrometry with hydride generation; SF, spectrofluorimetry.

The selenium content found in stomach was up to 30 times higher than the physiological level (31). In other organs, it exceeded physiological levels by 10–20 times with the relatively lowest value in lung tissue. The selenium concentration in blood was about 10 times higher than the normal selenium level in the blood of healthy people (10,32–35).

Conclusion

Based on the examinations performed, it can be stated that the HG-AFS method is a very useful analytical tool for determination of selenium in biological materials. In the case of human tissues collected after acute selenium poisoning, it can be considered as very competitive and complementary in relation to other analytical methods (SF, ET-AAS) applied routinely in this area for forensic purposes. Moreover, as it offers a low detection limit, a relatively dynamic range as well as very good accuracy and precision, HG-AFS can also serve in the reliable determination of selenium at very low concentrations; hence, it can be used to solve various toxicological and clinical problems connected with this element.

References

1. Barceloux DG. Selenium. Clin Toxicol 1999;37:145–72.
2. Högberg J, Alexander J. Selenium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. Handbook of the toxicology of metals. Amsterdam, the Netherlands: Elsevier, 2007;783–807.

3. Bedwal R, Nair N, Sharma M, Mathur R. Selenium—its biological perspectives. Med Hypotheses 1993;41:150–9.
4. Hill KE, Xia Y, Akesson B, Boeglin ME, Burk RF. Selenoprotein P concentration in plasma is an index of selenium status in selenium-deficient and selenium supplemented Chinese subjects. J Nutr 1995;126: 138–45.
5. Clark RF, Strukle E, Williams SR, Manoguerra AS. Selenium poisoning from a nutritional supplement. JAMA 1996;275:1087–8.
6. Whanger PD. Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. J Trace Elem Electrolytes Health Dis 1992;6: 209–21.
7. Gasmi A, Garnier R, Galliot-Guilley M, Gaudillat C, Quarterenoud B, Buisine A, et al. Acute selenium poisoning. Vet Human Toxicol 1997;39: 304–8.
8. Lindberg I, Lundberg E, Arkhammar P, Berggren PO. Direct determination of selenium in solid biological materials by graphite furnace atomic absorption spectrometry. J Anal At Spectrom 1988;3:497–501.
9. Köppel C, Baudisch H, Byer KH, Kloppel I, Schneider V. Fatal poisoning with selenium dioxide. J Toxicol Clin Toxicol 1986;24:21–35.
10. Matoba R, Kimura H, Uchima E, Abe T, Yamada T, Mitsukuni Y, et al. An autopsy case of acute selenium (selenious acid) poisoning and selenium levels in human tissues. Forensic Sci Int 1986;31:87–92.
11. Lech T. Suicide by sodium tetraoxoselenate(VI) poisoning. Forensic Sci Int 2002;130:44–8.
12. Matek M, Blanuša M. Comparison of two methods for destruction of biological material for determination of selenium. Arh Hig Rada Toksikol 1998;49:310–5.
13. Tamari Y, Ohmori S, Hiraki K. Fluorometry of nanogram amounts of selenium in biological samples. Clin Chem 1986;32:1264–7.
14. Petterson J, Hansson L, Örnemark U, Olin Å. Fluorimetry of selenium in body fluids after digestion with nitric acid, magnesium nitrate hexahydrate, and hydrochloric acid. Clin Chem 1988;39:1908–10.
15. Maspherson AK, Sampson B, Diplock AT. Comparison of methods for the determination of selenium in biological fluids. Analyst 1988;113:281–3.
16. Knowles MK, Brodie KG. Determination of selenium in blood by Zeeman graphite furnace atomic absorption spectrometry using a palladium-ascorbic chemical modifier. J Anal At Spectrom 1988;3:511–6.
17. Matek M, Blanuša M, Grgcić J. Comparison of two methods using absorption spectrometry for determination of selenium in food. Arh Hig Rada Toksikol 1999;50:283–8.
18. Emteborg H, Bordin G, Rodriguez AR. Speciation of organic and inorganic selenium in a biological certified reference material based on microbore ion-exchange chromatography coupled to inductively coupled plasma atomic emission spectrometry via a direct injection nebulizer or coupled to electrothermal atomic absorption spectrometry. Analyst 1998;123:245–53.
19. Wietecha R, Kościelniak P, Lech T, Rymanowski M. Determination of selenium in human blood using atomic fluorescence spectrometry. Probl Forensic Sci 2002;52:21–36.
20. Wietecha R, Kościelniak P, Lech T, Kielar T. Simple method for simultaneous determination of selenium and arsenic in human hair by means of atomic fluorescence spectrometry with hydride generation technique. Microchim Acta 2005;149:137–44.
21. Wietecha-Posłuszny R, Dobrowolska J, Kościelniak P. Method for determination of selenium and arsenic in human urine by atomic fluorescence spectrometry. Anal Lett 2006;39:2787–96.
22. Cai Y. Speciation and analysis of mercury, arsenic, and selenium by atomic fluorescence spectrometry. Trends Anal Chem 2000;19:62–6.
23. Rahman L, Corn WT, Bryce DW, Stockwell PB. Determination of mercury, selenium, bismuth, arsenic and antimony in human hair by microwave digestion atomic fluorescence spectrometry. Talanta 2000;52: 833–43.
24. Gámiz-Gracia L, Luque de Castro MD. Determination of selenium in nutritional supplements and shampoos by flow injection-hydride generation-atomic fluorescence spectrometry. Talanta 1999;50:875–80.
25. Ingrao G, Belloni P, Di Pietro S, Santaroni GP. Levels of some trace elements in selected autopsy organs, and in hair and blood samples from adult subjects of the Italian population. Biol Trace Elem Res 1990;26:699–708.
26. Turner J, Hill SJ, Evans EH, Fairman B. The use of ETV-ICP-MS for the determination of selenium in serum. J Anal At Spectrom 1999;14:121–6.
27. Lobinski R, Edmonds JS, Suzuki KT, Uden PC. Species-selective determination of selenium compounds in biological materials. Pure Appl Chem 2000;72:447–61.
28. Tinggi U, Gianduzzo T, Francis R, Nicol D, Shahin M, Scheelings P. Determination of selenium in red blood cells by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion. J Radioanal Nucl Chem 2004;259:469–72.
29. Machát J, Otruba V, Kanický V. Matrix interferences in determination of selenium by inductively coupled plasma—atomic emission spectrometry. Proceedings of the 2002 Winter Conference on Plasma Spectrochemistry; 2002 Jan 6–12; Scottsdale, AZ. Hadley, MA: ICP Information Newsletter, Inc., 2002;224–5.
30. Recknagel S, Brätter P, Tomiak A, Rösick U. Determination of selenium in blood serum by ICP-OES including an on-line wet digestion and Se-hydride formation procedure. Fresenius J Anal Chem 1993;346:833–6.
31. Wietecha-Posłuszny R, Dobrowolska-Iwanek J, Kościelniak P, Zagrodzki P. Determination of selenium as a biomarker of thyroid cancer by HG-AFS method. Acta Chim Slov 2009;56:441–6.
32. Oster O, Schmiedel G, Prellwitz W. The organ distribution of selenium in German adults. Biol Trace Elem Res 1988;15:23–44.
33. Yoshinaga J, Matsuo N, Imai H, Nakazawa M. Interrelationship between the concentrations of some elements in the organs of Japanese with special reference to selenium-heavy metal relationships. Sci Total Environ 1990;91:127–40.
34. Kraus T, Quidenus G, Schaller KH. Normal values for arsenic and selenium concentrations in human lung tissue. Arch Environ Contam Toxicol 2000;38:384–9.
35. Kinova L, Penev I. Selenium in organs and hair. Trace Elem Med 1990;7:155–6.
36. Miller JN, Miller JC. Statistics and chemometrics for analytical chemistry, 5th edn. Harlow, UK: Pearson, Prentice-Hall, 2005.

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