The use of definitive methods based on radiochemical neutron activation analysis for the determination of selenium and arsenic in materials used in proficiency testing

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Abstract. This article describes the use of two definitive methods for the determination of Se and As traces in biological materials intended for proficiency testing. Radiochemical neutron activation analysis (RNAA) definitive methods are based on reactor neutron activation with a very selective and quantitative post-irradiation separation of the indicator radionuclide by column chromatography, followed by γ-ray spectrometric measurement. Each of these methods has detection limit of the order of ng·g^{-1} or better, and yields accurate results. The expanded standard uncertainty for both methods is ca. 4%. Definitive RNAA methods were used for establishing assigned values for Se and As in materials employed in proficiency testing of "Plants" series.

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Introduction

Many important aspects of human activities depend on the reliability of the results of chemical measurements. Hence, the problem of quality of analytical results and particularly in trace analysis is very important. In spite of considerable development of all instrumental analytical techniques, the problem of accuracy in inorganic trace analysis still exists. From the comparison of individual instrumental techniques it could be concluded that no technique is an ideal one and each possesses some limitations. This could be seen from results provided during organization of international comparisons aimed at the determination of trace elements in biological matrices. The Institute of Nuclear Chemistry and Technology (INCT) as the producer of certified reference materials is the organizer of open worldwide interlaboratory comparisons. In case of several trace elements, data obtained from participants are not sufficient for assigning the certified values. For one of the last two materials: Corn Flour (INCT-CF-3) only 7 participants out of 92 total have delivered results for Se [16]. Moreover, the spread of results was very substantial (e.g. for Soya Bean Flour (INCT-SBF-4) the results for As varied between 0.22 ng·g^{-1} and 140 ng·g^{-1}) [17]. The position of neutron activation analysis (NAA) among the analytical techniques is still strong despite some weaknesses like the necessity of having access to a nuclear reactor, long analysis time etc. and NAA is without doubt a valuable method in inorganic trace analysis, especially important in certification of reference materials and solving problems with quality control/quality assurance (QC/QA). The highly accurate (definitive) methods

based on radiochemical NAA (RNAA) developed in this Institute have the potential to be termed primary methods of measurement PMM [8, 13]. These methods can be used for checking the accuracy of routine analytical methods and may be a valuable tool in the process of certification of reference materials or proficiency test (PT) samples. Definitive methods are being elaborated for elements essential or toxic for humans. In the past, methods for determination of Cu [9], Co [6], Mo [5] and Cd [19] have been elaborated in this Laboratory. Selenium is an essential element for mammals, their health is strongly dependent on excess or deficit of this element [18, 24]. Selenium deficiency may contribute to development of a form of heart disease, hypothyroidism, and weakening of immune system. On the other hand, high blood levels of selenium (greater than 1 mg·g^{-1}) can result gastrointestinal upsets, hair loss, fatigue, irritability, and mild nerve damage.

Arsenic is one of the most toxic elements that can be found on earth [1, 25]. Humans may be exposed to arsenic through food, water and air. Exposure may also occur through skin contact with soil or water that contains arsenic. Toxicity of As is strongly dependent on its form; inorganic forms are more toxic than the organic ones. Exposure to arsenic can cause various health effects, such as irritation of the stomach and intestines, decreased production of red and white blood cells, skin changes and lung irritation. It is suggested that the uptake of significant amounts of arsenic can intensify the chance of cancer development, especially the cancer of skin, lung, liver and lymphatic cancer. Finally, inorganic arsenic can damage DNA. In view of selenium and arsenic properties, it is necessary to have accurate and reliable methods for the determination of these elements in different types of biological samples. The purpose of this work was to demonstrate the usefulness of new definitive methods for the determination of trace amounts of Se and As in biological samples used in proficiency testing.

Experimental

Reagents, standards, radioactive tracers

All reagents were of highest grade available commercially. 18 MΩ·cm grade water from Milli-ORG Millipore Co purification system was used throughout. Amberlite XAD4 20–50 mesh (Rohm and Haas Co.) was ground and sieved to get a fraction of particle size of 0.10 mm ≤ φ ≤ 0.12 mm. Dowex 50 WX4 [H$^+$], 100–200 mesh (Serva) and Bio-Beads SM-2, 200–400 mesh (Bio-Rad Laboratories), were used as received. Column filling for extraction chromatography was prepared by suspending chosen support in a solution of appropriate *o*-diamine in a water-ethanol mixture (1:1). The solution was evaporated at a temperature of approximately 60°C, with occasional stirring to obtain coloured, dry powder. All used solutions were prepared by weigh. Standard of selenium was obtained from 99.999 ± 0.001% SeO$_2$, (Sigma) by weighing an appropriate amount of the oxide, dissolving in water, and weighing the solution obtained. Selenium standards for the irradiation were prepared by weighing aliquots of standard solution in

PE capsule and evaporated to dryness before encapsulation. The following radioactive tracers were used in elaboration and optimization of separation scheme: ^{75}Se ($T_{1/2}$ = 120 d), ^{59}Fe ($T_{1/2}$ = 44.5 d), ^{51}Cr ($T_{1/2}$ = 27.7 d), ^{181}Hf ($T_{1/2}$ = 42.4 d), ^{46}Sc ($T_{1/2}$ = 83.8 d), ^{86}Rb ($T_{1/2}$ = 18.7 d), ^{65}Zn ($T_{1/2}$ = 244 d), ^{133}Ba ($T_{1/2}$ = 10.5 y), ^{60}Co ($T_{1/2}$ = 5.27 y), ^{134}Cs ($T_{1/2}$ = 2.06 y), ^{73}As ($T_{1/2}$ = 80 d), ^{122}Sb ($T_{1/2}$ = 65 h), ^{186}Re ($T_{1/2}$ = 3.8 d). All tracers were prepared by neutron irradiation of spectrally pure oxides or salts (mostly nitrates) in the Polish nuclear reactor MARIA, except for ^{73}As which was produced in the Kraków cyclotron AIC 144 by the ^{nat}Ge(p,xn)$^{73-75}$As reaction.

Apparatus

To prepare samples, CRMs and standards for irradiation, calibrated analytical and micro-analytical balances Sartorius MC5 and Sartorius BP221S were used. To digest samples, a microwave high pressure system (Plazmatronika, Poland) was used. To perform gamma-ray spectroscopic measurements the following detectors were used:

- 180 cm^3 HPGe well-type (Canberra) with associated electronics (resolution 2.09 keV for 1332 keV ^{60}Co line, efficiency ca. 30%), coupled to a multichannel analyzer TUKAN (The Andrzej Soltan Institute for Nuclear Studies, Świerk, Poland), and
- 255 cm^3 HPGe well-type detector (Canberra) with associated electronics (resolution 2.15 keV for 1332 keV ^{60}Co line, efficiency ca. 55%), coupled to a multichannel analyzer and spectroscopy software Genie-2000 (Canberra).

High accuracy (definitive) methods

The samples of biological material, elemental standards, CRMs and blank were placed in polyethylene (PE) containers and irradiated in the MARIA reactor at Świerk, at a neutron flux of 10^{14} n·cm^{-2}·s^{-1} for one hour, and cooled for appropriate cooling time. Then, the samples were acid digested using a high-pressure microwave system. After decomposition, the samples were subjected to separation procedure. The separated radionuclides were measured by means of a gamma-ray spectrometer.

Results and discussion

High accuracy definitive methods

According to Uriano and Gravatt definition [23] and its contemporary version given by IUPAC [4], definitive methods of chemical analysis are those that have valid and well-described theoretical foundation, have been experimentally evaluated so that reported results have negligible systematic errors, and have high levels of precision. Definitive methods performed in the INCT are based on radiochemical neutron activation analysis involving selective and quantitative post-irradiation separation of the analyte by column chromatography followed by gamma-ray spectrometric measurements.

Elaboration of new definitive method has to be done according to several rules formulated previously:

– A single-element method based on neutron activation with selective and quantitative post-irradiation isolation of analyte (confirmed by tracer experiments) is employed;
– All potential sources of errors starting from sampling and sample digestion (dissolution) up to gamma spectrometric measurements should be identified at the stage of elaborating the method and removed or appropriate corrections should be introduced into the procedure;
– Whenever possible, the visual control of the correctness of the analytical procedure (colour of determined ion or its complex) should be used;
– At least two standards should be irradiated, one of which has passed through the whole radiochemical procedure, and the other is measured directly. Normalized analytical signals (with uncertainties) of the two standards, should overlap;
– Residual blank should be measured in each set of determinations.

It is very important, that no result is automatically classified as obtained by definitive method but first it is being checked if a series of criteria have been fulfilled [8, 13].

The main assumption during elaboration of definitive method is selective and quantitative isolation of analyte. Three ortho-diamine extractants (2,3-diaminonaphtalene, 3,3'-diaminobenzidine and 4-nitro-phenyldiamine) supported on appropriate sorbents were tested for selective separation of selenium. Column experiments demonstrated that, 3,3'-diaminobenzidine supported on Amberlite XAD-4 was the most beneficial column filling [12]. In the case of arsenic, it is well recognized that arsenic in the arsenate form is strongly sorbed by iron oxides and hydroxides such as goethite and ferrihydrite. Because the hydrated Fe(III) oxide is not suitable for column usage due to excessive pressure drop and column blockage, preparation of a special sorbent with Fe(III), being referred to henceforth as hybrid ion exchanger (HIX) was initiated and realized following some data found in the literature [2, 7]. To insure high selectivity and purity of isolated analyte fraction, the column with strongly acidic cation-exchanger Dowex 50 WX4 [H$^+$] was used in each separation scheme. The procedure is based on the fact that Se and As present in anionic form are not adsorbed by cation exchangers from dilute solutions of mineral acids, whereas interfering elements are strongly retained under these
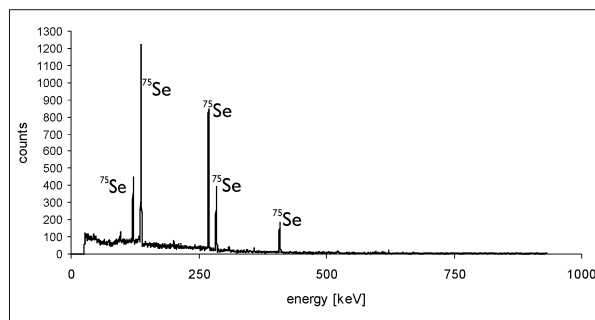
Fig. 1. Gamma-ray spectrum of selenium fraction obtained from 100 mg NBS 1577 Bovine Liver; measurement time: 500 s.
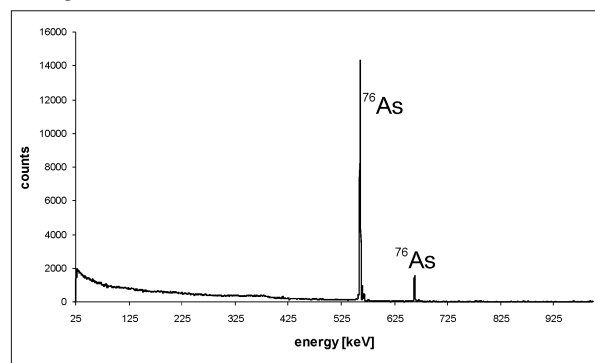
Fig. 2. Gamma-ray spectrum of arsenic fraction obtained from 100 mg NBS 1566a Oyster Tissue; measurement time: 1000 s.

conditions. Figure 1 (for Se) and Fig. 2 (for As) show, that radionuclide purity of both selected analytes is very high. Tracer experiments confirmed practically 100% efficiency of isolation.

One of the requirements of a definitive method is the comparison of the normalized analytical signals from two standards, one of which has passed through the whole radiochemical procedure and the other which has been measured directly. The activity of standards together with their confidence limits overlapped in all the cases – the differences between them never exceeded 5%.

In the case of method for selenium determination, visual control of the separation process is possible. During preconcentration of selenium traces on a column with 3,3'-diaminobenzidine, the yellow ring originated from piazselenol formed allows for visual control of the process.

The accuracy of the devised methods was demonstrated by analyzing biological CRMs, the obtained results are reported in Table 1.

Table 1. Results of the determination of As and Se in the certified reference materials

Element	Certified reference material	Certified value and its confidence limits	Arithmetic mean and its confidence limits*
As	Mixed Polish Herbs, INCT-MPH-2	191 ± 23 ng·g^{-1}	180 ± 15 ng·g^{-1}
	Lichen, IAEA-336	630 ± 82 ng·g^{-1}	625 ± 30 ng·g^{-1}
Se	Rice Flour, NBS 1568	370 ± 40 ng·g^{-1}	369 ± 27 ng·g^{-1}
	Bovine Liver, NIST 1577	1.10 ± 0.10 μg·g^{-1}	1.00 ± 0.06 μg·g^{-1}

* Results are presented in the form:

$$\bar{x} \pm \left[(t_{0.05} \cdot s) / \sqrt{n} \right],$$

where: $\bar{x}$– arithmetic mean; $t_{0.05}$ – parameter of t-Student's distribution for significance level α = 0.05 and $n - 1$ degrees of freedom; s – standard deviation; n – number of individual results.

Uncertainty budget evaluation

The uncertainty budget takes into account all possible sources of uncertainty starting from sampling up to measurements. Identification of the biggest contribution defines the most sensitive step in the elaborated procedure, and can initiate appropriate corrections of the procedure. In general, the sources of standard uncertainty (u_i) in RNAA can be grouped into the following categories [11, 21, 22]:

1. Preparation of samples, standards and monitors to the irradiation in the reactor, u_1, (weighing, purity of standards);
2. Irradiation in the neutron flux in a nuclear reactor, u_2, (homogeneity of neutron flux, nuclear reaction interferences, neutron self-shielding);
3. Radiochemical separations, u_3, (chemical yield);
4. Gamma-ray spectrometric measurements, u_4, (counting statistics, counting geometry differences, pulse pile-up losses, peak integration method).

Obviously, the contribution of the individual sources to the total uncertainty is not of the same importance. In the case of selenium, the biggest contribution into the whole uncertainty value results from radioactivity measurements of selenium traces and standards retained on the sorbent (differences in counting geometry). Contrary to the solutions, obtaining the same, well defined shape is more difficult in this case due to problems with homogenization of the sorbent. Moreover, the major contributions to the uncertainty were found from the counting statistics for standards and samples (total of 21.5%). In the case of arsenic, the biggest contribution to the uncertainty budget gives counting statistics of samples and standards. This is connected with a relatively short half-life time of ^{76}As (26.3 h). On the other hand, the contribution from several items is negligible (e.g. from the purity of standards – the purity of SeO_2 used to standard preparation was $99.999 \pm 0.001\%$ and the contribution calculated from rectangular distribution was 0.057%).

The uncertainty budget for selenium determination was calculated for NBS 1547 Peach Leaves with Se content 120 ± 10 ng·g^{-1}. Combined standard uncertainty amounts to 1.7%. The final result obtained by the definitive method with expanded uncertainty ($k = 2$) is equal to 127 ± 5 ng·g^{-1} [3]. The uncertainty budget for arsenic determination was calculated for CTA-OTL-1 Tobacco Leaves with As content of 539 ± 59 ng·g^{-1}. Combined standard uncertainty amounts to 1.7%. The final result obtained by the definitive method with expanded uncertainty ($k = 2$) is equal to 543 ± 19 ng·g^{-1} [2].

Proficiency testing PLANTS

Previous definitive methods were used to confirm certified values during certification process of new reference materials. Methods for selenium and arsenic determination were elaborated recently, and they were of use to determine assigned value in proficiency testing materials. Proficiency testing (PT) consist in the use of comparisons of test results or measurements to determine the performance of individual laboratories and to monitor the laboratory's performance. Participation in
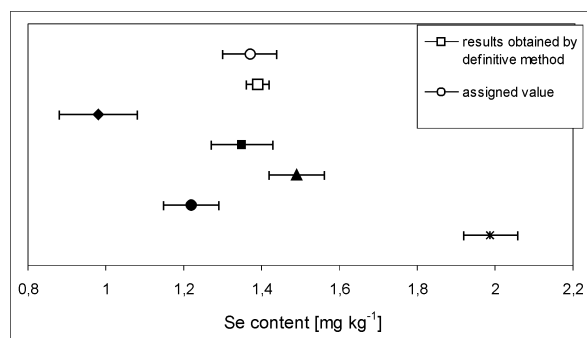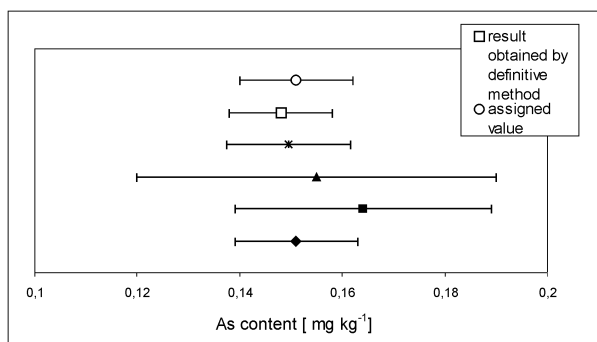

Fig. 3. Determination of selenium in dry mushroom powder (*Suillus bovinus*) by expert laboratories – comparison with the assigned value and with the result obtained by RNAA definitive method.

proficiency testing provides laboratories with an objective means of assessing and demonstrating the reliability of the data they are producing. The INCT has been involved as the organizer of Proficiency Tests "Plants". This programme is dedicated for Polish laboratories working in the fields of food and agriculture, environment and safety. Participants are asked to determine elements selected from the group of toxic and essential elements: As, Cd, Cr, Cu, Hg, Pb, Se, Zn in plant materials. The organizers have a possibility of using different approaches to evaluate the assigned value. In the INCT the assigned value is determined on the basis of results obtained from a few expert laboratories. In the spring 2006, the INCT organized a new PT "Plants 6: Determination of trace elements in dry mushrooms powder (*Suillus bovinus*)" [15]. Assigned value was calculated as the Huber robust mean obtained by the algorithm recommended by ISO 13528:2005 as "algorithm A" [10] and IUPAC protocol [20]. The standard uncertainty of the assigned value was estimated as:

$$(1) \qquad u_x = 1.23 \cdot \left(n \cdot \sqrt{\left(\sum_{i=1}^{n} u_i^2 \right)} \right)^{-1}$$

where: n – number of expert laboratories; u_i – standard uncertainty of the results of ith laboratory.

Assigned value with expanded uncertainty ($U, k = 2$) for selenium in the analyzed material was calculated as 1.37 ± 0.11 mg·kg^{-1}, whereas the result obtained by the definitive method was 1.39 ± 0.015 mg·kg^{-1}. Figure 3 shows the results obtained by other expert laboratories in comparison with the assigned value and with the result obtained by the definitive method. Following PT "Plants 7: Determination of trace elements in a dry mushroom powder (champignon)" was carried out in 2007 [14]. Also in this exercise, the result obtained by the RNAA definitive method 2.67 ± 0.18 mg·kg^{-1} and the assigned value 2.676 ± 0.120 mg·kg^{-1} agree very well. For determination of arsenic, the assigned value with expanded uncertainty ($U, k = 2$) in the analyzed material was calculated as 0.151 ± 0.010 mg·kg^{-1}, whereas the result obtained by the definitive method was 0.146 ± 0.012 mg·kg^{-1}. Figure 4 shows the results obtained by other expert laboratories in comparison with the assigned value and with the result obtained by the definitive method. As can be seen, the delivered

Fig. 4. Determination of arsenic in dry mushroom powder (champignons) by expert laboratories – comparison with the assigned value and with the result obtained by RNAA definitive method.

results are similar but some results show very high level of uncertainty.

There is strong evidence that determination of these two elements is still problematic for analysts. Only 21% (Plants 6) and 20% (Plants 7) of participants have delivered results for selenium. From the statistical evaluation (z-score, zeta-score and E_n numbers) it could be concluded that ca. 30% of them produced unacceptable results.

Conclusions

As can be seen, the elaborated definitive methods for selenium and arsenic determination deliver precise results with very low levels of uncertainty, which is characteristic of primary methods of measurement. The expanded uncertainty of 4% makes these methods comparable with isotope dilution mass spectrometry. In the case of monoisotopic elements, like arsenic, ID-MS cannot be used and RNAA seems to be the only very accurate method available. The use of definitive methods proved to be very helpful in the determination of assigned values in proficiency tests. In general, it could be concluded that these methods are not dedicated to routine analysis, but they are recommended for checking the accuracy of other techniques of inorganic trace analysis and for certification of new reference materials as well as for assigning values in proficiency tests.

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