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SHORT COMMUNICATION

CERTIFIED REFERENCE MATERIALS FOR QUALITY CONTROL OF MERCURY AND SELENIUM DETERMINATION IN FOOD

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Several certified reference materials were used for quality control of mercury and selenium determination in food through different analytical procedures and techniques. The materials were selected with respect to different composition of matrix and levels of certified values. The results agreed well with the certified values for all analysed materials.

Key words:
closed system digestion, open system digestion, sample preparation, spectrofluorometry, vapour atomic absorption spectrometry

The problem of quality assurance (QA) of elemental analysis is of crucial importance for proper assessment of food quality. Food is the main source of macro-, micro-, and trace elements which may play different role in human organism, that is, nutritional, essential, and/or toxic. The levels of these elements are among the factors that characterize the safety and quality of foodstuffs. For that reason, regulations in many countries including Poland define permissible levels of toxic or potentially toxic elements in food products, raw materials, and food additives (1). Reliable and accurate data are hence necessary to verify compliance with regulations or other specifications and make sound decisions. These data can be achieved by implementation of a suitable QA system through laboratories involved in the analysis of food (2, 3).

A targeted use of certified reference materials (CRMs) is one of approaches to the quality control (QC) of food analysis (4). CRMs are used in our laboratory to evaluate the accuracy of analytical procedures and compare different digestion procedures and different techniques of determinations. They are also used for internal quality control of routine food analysis, mainly of plant origin. In order to demonstrate the credibility of analytical results in all these areas of our activity we use several CRMs which are chosen with respect to the analyte, levels of certified values, and matrix composition.

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This paper summarizes the use of CRMs for QA of mercury and selenium determination in food. Both elements have been given much attention because of evident effects these have on human health. The first element is recognized as evidently toxic whereas the latter can be either toxic or essential to humans, depending on the concentration level (5–7). In general, both elements occur in very low quantities in food of plant origin, which in turn requires sensitive and accurate methods for their determination. Numerous techniques have been developed for determination of low mercury and selenium levels in food and environmental samples (8, 9). However, only neutron activation analysis and, additionally, fluorometry for selenium meet the requirements for recommended determination methods. Yet, these techniques have some limitations. NAA requires expensive equipment and is as time-consuming as fluorometry. Among different techniques, cold vapour atomic absorption spectrometry (CVAAS) and hydride generation vapour atomic absorption spectrometry (HGAAS) are the most widely used techniques for the respective mercury and selenium determination. However, these techniques require complete decomposition of organic matter that could be achieved under vigorous conditions that involve the use of strong acid mixtures with or without addition of other oxidants at elevated temperature and pressure (8). The recent microwave digestion technique has provided some advantages such as the use of low oxidants, short time, and more controllable conditions of digestion. Sample preparation is equally important in the analytical procedure for mercury and selenium determination in food using CRMs as is the condition of analytical instruments, since both steps may bring losses and contamination (5, 8–10). We have, therefore, examined both steps by verifying mercury determination with CVAAS after microwave digestion, by comparing HGAAS technique with spectrofluorometric method for selenium determination, and by comparing the two methods of digestion in the open (heating block) and the closed system (microwave oven) for determination of selenium.

MATERIALS AND METHODS

The conditions of the sample digestion procedures and determination of mercury and selenium were optimised and described earlier (11).

Instrumentation, reagents, and test samples

Instruments included a microwave oven MDS-2000 (CEM), a heating block with a temperature controller (Institute for Land Reclamation and Grassland Farming, Experimental Department, Poland), a fully automated mercury analysis system (LDC), a double beam fluorescence spectrophotometer (Perkin Elmer Model 512), atomic absorption spectrophotometer PU9200 (Philips) equipped with a PU9360 continuous flow vapour, and a water bath.

We used the following reagents: 70% nitric acid, 70% perchloric acid, 30% hydrochloric acid, 98% sulphuric acid, 0.6% sodium borohydride ($NaBH_4$) in 0.5% NaOH, 5% tin chloride in 10% sulphuric acid, 2,3-diaminonaphthalene (DAN), 0.2% ethylenediaminetetraacetate (EDTA), cyclohexane, stock solutions of Hg and Se each at 1000

μg/ml, and double-distilled deionised water. All chemicals used were of analytical-reagent grade.

The CRMs used for the quality control of analytical procedures for mercury and selenium determination were obtained from Community Bureau of Standards (BCR, Brussels, Belgium) – BCR No 62 Olive Leaves, BCR No 189 Wholemeal Flour, CRM 281 Rye Grass, BCR No 185 Bovine Liver and BCR No 274 Single Cell Protein, National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards – NBS; Gaithersburg, USA) – NIST 1515 Apple Leaves, National Bureau of Standards Standard Reference Material (NBS SRM) 1568 Rice Flour, NBS SRM 1571 Orchard Leaves, NBS SRM 1572 Citrus Leaves, Comité Inter-Institutés (CII) – CII 875 Cabbage and CII 883 Carnation, Institute of Physics and Nuclear Techniques – CL-1 Cabbage Leaves (Polish CRM), and Institute of Nuclear Chemistry and Technology – Polish Certified Reference Material Oriental Tobacco Leaves CTA-OTL-1.

Sample preparation

Microwave digestion (closed system) for mercury determination: 0.3 g of a sample was weighed into a 100 ml PTFE digestion vessel, 3 ml of HNO_3 were added and then left overnight. The digestion procedure was carried out the following day through heating stages as follows: I – 30% of power, 2 atm, 10 min; II – 80% of power, 4 atm, 10 min; III – 100% of power, 12 atm, 20 min.

The digestion in heating block (open system) for selenium determination: 0.3 g of a sample with 5 ml of a mixture of concentrated HNO_3-$HClO_4$ (3+1 v/v) in a digestion glass tube was left overnight. The sample was heated the following day in the heating block at 50 °C for 1 hour, at 70 °C for 6 hours, and at 125 °C for 12 hours.

The reduction of Se^{6+} to Se^{4+} was obtained by adding 2 ml of concentrated HCl to the digest and heating all at 125 °C for 30 minutes.

Microwave digestion (closed system) for selenium determination: 0.3 g of a sample was weighed into a 100 ml PTFE digestion vessel and 5 ml of HNO_3 and 2 ml of HCl were added. The sample was left overnight and the following day it was heated by microwave radiation in three stages: at 2 atm of pressure, 60% of power for 10 minute then at 5 atm of pressure, 80% of power for 10 minute and then at 12 atm of pressure, 100% of power for 20 min. The final solution was transferred into 25 ml volumetric flask and diluted to volume with water.

The reduction of Se^{6+} to Se^{4+} was done by adding 2 ml of concentrated HCl to 10 ml of the sample solution and heating all in a water bath for 30 min.

Each sample digestion procedure was carried out with a blank and the highest working standard solution in three replicates.

Mercury and selenium measurements

Mercury was determined using a fully automated mercury analysis system (FAMAS). Instrumental parameters were as follows:

Mercury Monitor 3200 detector:
- range: 0.005 AU
- response time: 5 s
- dryer tube: packed with magnesium perchlorate

Reagents:
– reducing solution: $SnCl_2$ in H_2SO_4
– acid solution: 2% nitric acid
Analysis time: 5 min.

Determination of selenium by spectrofluorometry (Se^{4+}– DAN complex formation): 25 ml of EDTA solution, 20 ml of water and 5 ml of DAN solution were added to the digested solutions after reduction in the heating block. The solution was mixed and placed in a pre-heated to 60 °C water bath for 40 min. After cooling to room temperature, the solution was transferred to the separation funnel and 5 ml of cyclohexane were added. The content was shaken vigorously for 1 minute and then left to separate for 5 minutes. The cyclohexane layer was transferred to a 5 ml quartz cuvette and selenium was immediately determined, λ_{ex}–375 nm, and λ_{em}–520 nm.

Determination of selenium by HGAAS: in solutions after heating block and microwave digestion and the reduction of Se^{6+} to Se^{4+}, selenium was determined using a continuous flow vapour system. Instrumental parameters were as follows:
Blank – 5% HCl
Reductant – $NaBH_4$ solution
Argon flow – 300 ml/min
Stabilization time – 30 s
Baseline time – 40 s
Background correction – off

Analytical characteristics of the methods

Calibration range: the linear range for mercury determination by CVAAS was 0–5 ng/ml while for selenium determination by HGAAS and spectrofluorometry it was 0–40 ng/ml and 0–300 ng, respectively.

Detection limits for mercury and selenium were established by the analysis of the reagent blanks that underwent the total analytical procedure (3 times the standard deviation of 10 blank determinations). These were 4.4 pg/ml for mercury determination by CVAAS and 0.24 ng/ml and 1.5 ng for the respective selenium determination by HGAAS and spectrofluorometry.

The precision of the methods was established from 6 repeated determinations of 5 certified reference materials for mercury and selenium content. Depending on the element, its concentration level in the CRM, and the type of matrix, the coefficient of variations varied from 1.5 to 13.4% for mercury determination by CVAAS, from 4.0 to 12.6% for selenium determination by HGAAS, and from 4.5 to 13.8% for selenium determination by spectrofluorometry.

RESULTS AND DISCUSSION

The verification of the analytical procedure for mercury determination was carried out using five CRMs of plant origin and one of animal origin. The certified values of mercury ranged from 0.0060 to 0.280 μg/g. Our results agreed well with the certified

values for all analysed materials (Table 1). This method has successfully been used for routine analyses of mercury in food of plant and animal origin that are performed in our laboratory. In order to control the precision and accuracy of the method on a

Table 1 *Analytical data for mercury determination in certified reference materials by CVAAS*

Certified reference material	Mercury content, in µg/g	
	Certified value	Found
NBS SRM 1568 Rice Flour	0.006 ± 0.001[a]	0.007 ± 0.001[c]
BCR 281 Rye Grass	0.021 ± 0.002[b]	0.021 ± 0.001[c]
NIST 1515 Apple Leaves	0.044 ± 0.004[b]	0.048 ± 0.001[c]
BCR 185 Bovine Liver	0.044 ± 0.003[b]	0.043 ± 0.002[c]
NBS SRM 1572 Citrus Leaves	0.080 ± 0.020[a]	0.084 ± 0.003[c]
BCR 62 Olive Leaves	0.280 ± 0.020[a]	0.291 ± 0.013[c]

[a] Certified value ± the estimated uncertainty that represents the combined effects of method imprecision, possible systematic errors among methods and material variability (but in no case less than 95% confidence limit)
[b] Certified value ± 95% confidence interval of the mean value
[c] Mean ± standard deviation at 95% confidence limit for N = 6

long-term basis, the CRM NIST 1515 Apple Leaves was used as control material on every analysis. The obtained results fell within ±2 times the 95% confidence interval of the certified mean value and they are presented on the control chart (Figure 1).

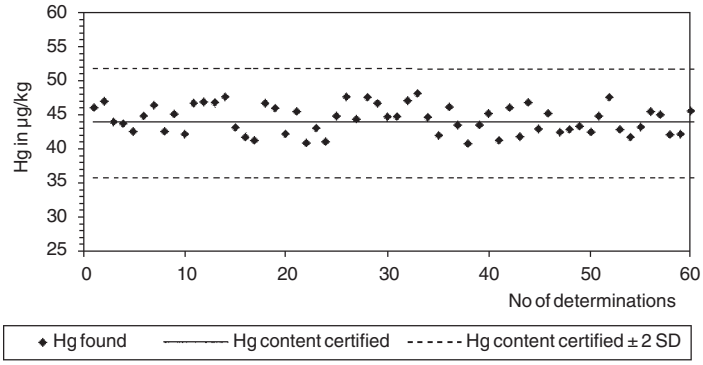

Figure 1 *NIST 1515 Apple Leaves control chart for mercury determinations in food by CVAAS technique in 1997*

The verification of the analytical procedure for selenium determination by HGAAS technique included the comparison with spectrofluorometric method, already in use in our laboratory, and the comparison of two types of digestion. The comparisons

involved five CRMs with certified values ranging from 0.08 to 1.03 μg/g and the results are presented in Tables 2 and 3. No significant differences were observed between the methods of sample pre-treatment and selenium determination.

Table 2 *Comparison of methods for selenium determination by HGASS and spectrofluorimetry after conventional digestion*

Certified reference material	Selenium content, in µg/g		
	Certified value	HGAAS	Spectrofluorometry
NBS SRM 1571 Orchard Leaves	0.08 ± 0.01[a]	0.08 ± 0.01[c]	0.085 ± 0.004[c]
CRM 189 Wholemeal Flour	0.132 ± 0.010[b]	0.13 ± 0.01[c]	0.122 ± 0.008[c]
CTA-OTL-1 Oriental Tobacco Leaves	0.153 ± 0.018[b]	0.16 ± 0.03[c]	0.159 ± 0.023[c]
CL-1 Cabbage Leaves	0.20 ± 0.03[b]	0.19 ± 0.03[c]	0.182 ± 0.019[c]
CRM 274 Single Cell Protein	1.03 ± 0.05[b]	1.01 ± 0.03[c]	1.026 ± 0.011[c]

[a] Certified value ± the estimated uncertainty that represents the combined effects of method imprecision, possible systematic errors among methods and material variability (but in no case less than 95% confidence limit)
[b] Certified value ± 95% confidence interval of the mean value
[c] Mean ± standard deviation at 95% confidence limit for N = 6

Table 3 *Comparison of conventional and microwave methods of digestion for selenium determination by HGAAS*

Certified reference material	Selenium content, in µg/g		
	Certified value	Digestion	
		Microwave	Conventional
NBS SRM 1571 Orchard Leaves	0.08 ± 0.01[a]	0.08 ± 0.01[c]	0.08 ± 0.01[c]
CRM 189 Wholemeal Flour	0.132 ± 0.010[b]	0.12 ± 0.01[c]	0.13 ± 0.01[c]
CTA-OTL-1 Oriental Tobacco Leaves	0.153 ± 0.018[b]	0.15 ± 0.01[c]	0.16 ± 0.03[c]
CL-1 Cabbage Leaves	0.20 ± 0.03[b]	0.20 ± 0.02[c]	0.19 ± 0.03[c]
CRM 274 Single Cell Protein	1.03 ± 0.05[b]	1.07 ± 0.04[c]	1.01 ± 0.03[c]

[a] Certified value ± the estimated uncertainty that represents the combined effects of method imprecision, possible systematic errors among methods and material variability (but in no case less than 95% confidence limit)
[b] Certified value ± 95% confidence interval of the mean value
[c] Mean ± standard deviation at 95% confidence limit for N = 6

The accuracy of the methods was additionally checked by the participation in the international proficiency testing program – IPE (International Plant Analytical Exchange), organized by Wageningen Agricultural University, the Netherlands. Figure 2 and 3 show the assessment of results obtained for mercury and selenium determination in 1997. Some results are missing, as they were not quantified by our laboratory or assessed by the organizer (12). In general, the obtained results were satisfactory because they did not exceed the z-score of ±2, that is, the lower warning limits.

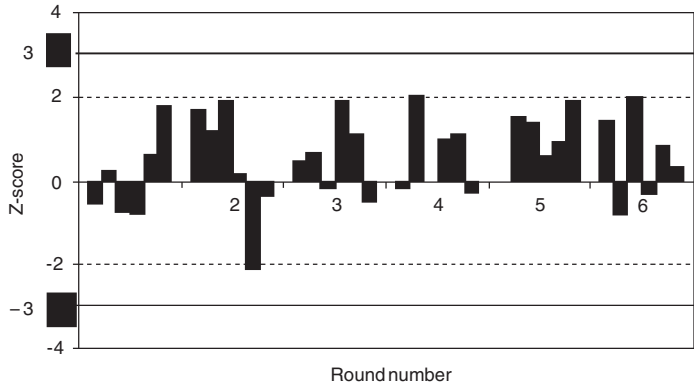

Figure 2 *Control chart for mercury determinations in dried plant materials by CVAAS during participation in IPE proficiency testing in 1997 (6 samples in 6 rounds a year)*

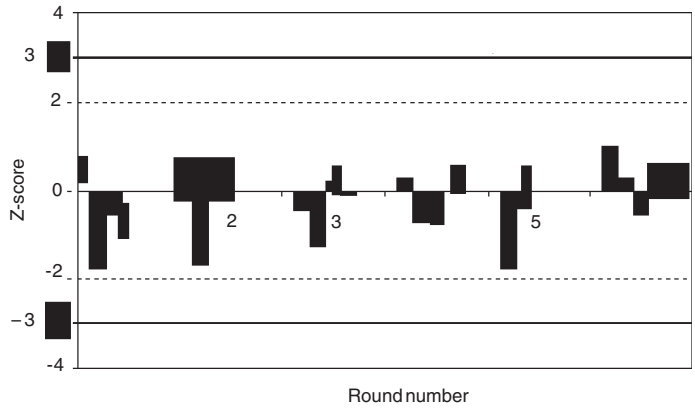

Figure 3 *Control chart for selenium determinations in dried plant materials by HGAAS during participation in IPE proficiency testing in 1997 (6 samples in 6 rounds a year)*

To conclude, the results of this study have shown that the methods of mercury and selenium determination using CVAAS and HGAAS after a suitable digestion procedure may guarantee achievement of satisfactory results. The use of CRMs in controlling the steps of and the whole analytical procedure may assure the achievement of results of required accuracy.

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Sažetak

OVJERENI REFERENTNI UZORCI MATERIJALA ZA KONTROLU KAKVOĆE ODREĐIVANJA ŽIVE I SELENIJA U HRANI

Upotrijebljeno je nekoliko ovjerenih referentnih uzoraka materijala u svrhu provjere metodoloških postupaka određivanja žive i selenija u hrani. Uzorci su odabrani na temelju spoznaja o vrstama matriksa očekivanih uzoraka za analizu, kao i mogućih koncentracija u uzorcima hrane. Priprema uzoraka uključivala je razgradnju otvorenim postupkom (kiselinama) i zatvorenima postupkom (u mikrovalnoj peći). Postignuti rezultati upućuju na dobro slaganje vrijednosti referentnih uzoraka.

Ključne riječi:
plinska atomska apsorpcijska spektrometrija, pripremanje uzoraka, razgradnja u otvorenom sustavu, razgradnja u zatvorenom sustavu, spektrofluorometrija

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