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SHORT COMMUNICATION

COMPARISON OF TWO METHODS USING ATOMIC ABSORPTION SPECTROMETRY FOR DETERMINATION OF SELENIUM IN FOOD

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This study describes and compares two methods of atomic absorption spectrometry (AAS) for the selenium (Se) analysis in food: electrothermal AAS (ET AAS) and hydride generation method of AAS (HG AAS). The accuracy of the two methods was established by analysing two biological reference materials: Wheat Flour 1567a and Bovine Liver 1577b from the National Institute of Standards and Technology, USA. Good agreement with certified values was obtained for both methods. The accuracy of ET AAS method is 109% and 103% for Wheat Flour and Bovine Liver, respectively. The respective accuracies for HG AAS method were 88% and 87%. The detection limit obtained for ET AAS was 1µg Se/L and for HG AAS 0.02 µg Se/L. The repeatability of ET AAS method was 5–11% and that of HG AAS 14–17%. Both methods are similar in accuracy and repeatability, but hydride generation (HG AAS) is more sensitive than graphite furnace technique (ET AAS) for determination of selenium in food.

Keywords:
accuracy, detection limit, certified values, electrothermal atomic absorption spectrometry, hydride generation method, repeatability

Concentrations of trace elements, including selenium, in biological material are determined by neutron activation analysis (NAA), inductively coupled plasma-mass spectrometry (ICP-MS), electrothermal atomic absorption spectrometry (ET AAS), fluorometry, or hydride generation atomic absorption spectrometry (HG AAS). Among these methods, NAA and ICP-MS are not readily accessible to many laboratories. Although HG AAS and fluorometry (1) are widely used, these require laborious and time-consuming pre-treatment, greater sample volume, and are generally more demanding. ET AAS, which is also widely used, requires a small sample volume even if several elements are to be determined. The problem with Se analysis using ET AAS

is that Se and some of its compounds are volatile and tend to evaporate during preatomisation. The other problem is that biological material contains high concentration of iron, phosphorus, and chlorides interfering with Se determination (2–5). This makes a matrix modifier necessary for the application of the electrothermal method (6–9). Literature commonly refers to ET AAS method for Se analysis in human serum without any digestion pre-treatment (10–12). In food or food commodities, Se is usually determined by HG AAS and fluorometry. There are few reports on Se determination in infant food (5, 6) and various certified reference materials (fish and cereals) (4, 13) by ET AAS method. The purpose of this study was to measure Se using the ET AAS method with a Pd matrix modifier after microwave digestion of various food items and to compare it with the HG AAS method.

MATERIAL AND METHODS

Instrumentation

Closed-vessel microwave digestion system (MDS-2000, 630 W), equipped with pressure monitoring option (maximum operating pressure, 13.8 bar), sample carousel, and advanced composite vessels (CEM Corp., Matthews, NC, USA) was used for digestion of food samples. Atomic absorption spectrometer (Varian AA300) was fitted with a graphite furnace (GTA 96), autosampler, selenium super lamp, and a deuterium lamp for background correction. Standard addition method was used for ET AAS measurements. Atomic absorption spectrometer (Perkin-Elmer 2380, Germany) with Mercury/Hydride System type 10 (MHS-10) and selenium electrodeless discharge lamp (EDL) was applied for the HG AAS method. Water was deionised with a Labconco (Water pro PS, USA) deioniser.

Chemicals

Deionised water of 0.06 μS/cm conductivity was used to prepare all solutions. Samples were digested with nitric acid, ultrapure grade (65%, Merck, Germany) and hydrogen peroxide *pro analysi* grade (30%, Kemika, Croatia). Hydrochloric acid, ultrapure grade (30%, Merck, Germany) and L-ascorbic acid ($C_6H_8O_6$, Sigma, USA) were used to reduce Se. Working stock standard (10 mg Se/ml) and working standard (40 μg Se/L) were prepared from high purity selenium stock solutions (1000±2 mg Se/L as SeO_2 in 0.5 mol/L HNO_3, Merck, Germany). Se-hydride was formed by addition of sodium tetrahydroborate (III) ($NaBH_4$, Kemika, Croatia). ET AAS measurement included Palladium chloride ($PdCl_2$, Fluka, Switzerland) as a matrix modifier and Triton X-100 (Packard, USA).

Certified Standard Reference Materials included Wheat Flour 1567a and Bovine Liver 1577b, both from the National Institute of Standards and Technology (NIST, USA). Food items measured either by ET AAS or by HG AAS were chicken, liver, tuna, hake, eggs, garlic, bread, wheat flour, and milk.

Sample preparation and measurement

The samples were digested with concentrated nitric acid in a microwave oven, as described elsewhere (14). Three g of fresh food or 0.2 g of lyophilised standard reference material Bovine Liver or Wheat Flour were mixed with 3–5 ml of concentrated HNO_3. After digestion, the samples were adjusted to 10 ml with deionised water. Up to that point the procedure was identical for both methods of Se measurement. For ET AAS, selenium was then reduced with 1 ml of 25 mmol/L ascorbic acid (with one drop of Triton X-100 added), the solution was added 1 ml of 9.4 mmol Pd/L (as $PdCl_2$ in 65 mmol/L HCl) which served as a matrix modifier, and the mixture was measured within few minutes. The volume of the sample that was dispensed into the graphite tube was 10 μl. The ashing temperature was 1000 °C and the atomisation temperature was 2600 °C, with nitrogen and argon as inert gases. Table 1 shows the optimal furnace settings. The instrument readings for the wavelength and slit width were 196 nm and 1.0 nm, respectively.

Table 1 *Optimal furnace programme settings for measurements by electrothermal atomic absorption spectrometry (ET AAS)*

Step (No)	Temperature (° C)	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	85	5.0	3.0	Nitrogen	No
2	95	40.0	3.0	Nitrogen	No
3	120	10.0	3.0	Nitrogen	No
4	1,000	5.0	3.0	Nitrogen	No
5	1,000	5.0	3.0	Nitrogen	No
6	1,000	2.0	0.0	Argon	No
7	2,600	1.0	0.0	Argon	Yes
8	2,600	2.0	0.0	Argon	Yes
9	2,600	2.0	3.0	Nitrogen	No

The other method used for comparison was HG AAS described by *Bye and Lund* (15). For HG AAS measurements, Se was reduced to four oxidation state with HCl (5 mol/L) at 60 °C for 30 minutes. The volume of the sample which was taken for Se reduction was up to 5 ml, depending on Se concentration. After the reduction, all Se was converted to hydrogen selenite with 3% $NaBH_4$ in 1% NaOH. The instrument readings for the wavelength and slit width were 196 nm and 2.0 nm, respectively. The total volume in the reaction vessel was 50 ml. Certified standards of Wheat Flour and Bovine Liver were used to test the accuracy of each method. The limit of detection (LOD) was calculated according to the below formula (16):

$$LOD = \frac{Sx3xC_{(standard)}}{\bar{X}}$$

where $C_{(standard)}$ is the average concentration of the lowest standard [$C_{(standard)}$=2 μg Se/L (ET AAS) and 0.4 μg Se/L (HT AAS)], $\bar{X}$ is the arithmetic mean of ten measurements of the lowest standard, and S is the corresponding standard deviation. The repeatability

(expressed as % relative standard deviation, RSD) of the whole procedure was checked with 4–10 replicates of the same food item, digested and measured on the same day.

RESULTS AND DISCUSSION

The results of certified standard measurement showed (Table 2) that the recovery of ET AAS method was 109% and 103% for Wheat Flour and Bovine Liver, respectively. The respective recoveries for HG AAS method were 88% and 87%. The detection limit obtained for ET AAS was 1 μg Se/L and for HG AAS 0.02 μg Se/L. The substantial difference in detection limits (five times in favour of HT AAS) is due to much higher aliquot of samples which can be taken for analysis in the latter method (10 μl in ET AAS compared to 5 ml in HG AAS method). The repeatability of ET AAS method presented as RSD in Table 2 was 5–11% and that of HG AAS 14–17% for different food commodities. Under the established optimum conditions, the calibration curve showed good linearity for ET AAS in the range 2–15 μg Se/L and for HG AAS in the range 10–50 ng Se/L. Samples of different food items with concentration of Se higher than 0.05 μg Se/g wet weight such as meat, offal, fish, eggs, and garlic, were easily measured by the ET AAS method. However, lower concentrations of Se like those found in bread, wheat flour, or milk had to be measured by HG AAS.

Table 2 *Recovery and repeatability (RSD) of two atomic absorption spectrometric methods for selenium measurement in food: electrothermal (ET AAS) and hydride generation (HG AAS)*

Biological material	Certified value (µg/g)	*Measured value (µg/g)	Recovery (%)	RSD (%)	Method
Wheat flour 1567a,	1.1 ± 0.2	1.2 ± 0.09	109.1	7.5	ET AAS
NIST		0.97 ± 0.17	88.2	17.5	HG AAS
Bovine liver 1577b,	0.73 ± 0.06	0.75 ± 0.08	102.7	10.6	ET AAS
NIST		0.63 ± 0.09	86.6	14.3	HG AAS
Chicken fried	–	0.328 ± 0.019	–	5.8	ET AAS
Bovine liver fried	–	0.121 ± 0.011	–	9.0	ET AAS
Tuna canned	–	0.971 ± 0.073	–	7.5	ET AAS
Hake fresh	–	0.248 ± 0.015	–	6.0	ET AAS
Egg white raw	–	0.053 ± 0.004	–	7.5	ET AAS
Egg yolk raw	–	0.262 ± 0.021	–	8.0	ET AAS
Garlic raw	–	0.141 ± 0.007	–	4.9	ET AAS
Bread half white	–	0.070; 0.062	–	–	HG AAS
Wheat flour white	–	0.048; 0.033	–	–	HG AAS
Milk (2.8% fat)	–	0.008; 0.008	–	–	HG AAS

*The results are presented as arithmetic means ± standard deviations of 10 replicates (ET AAS) or 4 replicates (HG AAS) of the same biological sample. Both results are given where there were only two measurements.
RSD–relative standard deviation.

To conclude, the two methods showed comparable accuracy and repeatability, but different detection limits. Biological material which accumulates Se can be successfully measured by the ET AAS method with Pd as a matrix modifier. The only difference between the method described for serum in the literature (10–12) and our modification was in the higher concentration of $PdCl_2$, in direct mixing with digested sample, and in immediate measurement. However, HG AAS more easily measures samples with lower Se concentrations, because of higher sample volumes which can be taken for hydride formation.

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Sažetak

USPOREDBA DVIJU METODA ATOMSKOAPSORPCIJSKE SPEKTROMETRIJE ZA ANALIZU SELENIJA U HRANI

U ovom radu uspoređene su i opisane dvije metode atomskoapsorpcijske spektrometrije (AAS) za određivanje selenija (Se) u hrani: elektrotermalna metoda AAS (ET AAS) i hidridna tehnika AAS (HT AAS). Razaranje uzoraka za obje metode provedeno je u mikrovalnom uređaju (CEM, MDS-2000) koncentriranom dušičnom kiselinom. Mjerenju Se ET AAS metodom prethodi redukcija elementa askorbinskom kiselinom (u koju je dodan Triton X-100). Za testiranje točnosti i ponovljivosti metoda primijenjeni su standardni referentni materijali goveđe jetre i pšeničnog brašna (Bovine Liver 1577b i Wheat Flour 1567a, NIST, SAD). Granica detekcije i ponovljivost rezultata mjerenja određeni su za obje metode. Točnost određivanja Se ET AAS metodom za referentni materijal goveđa jetra i pšenično brašno iznosi 109%, odnosno 103%. Točnost mjerenja Se metodom HT AAS u istim materijalima iznosi 88%, odnosno 87%. Granica detekcije mjerenja Se metodom ET AAS je 1 µg Se/L, a metodom HT AAS 0,02 µg Se/L. Ponovljivost rezultata mjerenja ET AAS metode kreće se od 5 do 11%, a HT AAS metode od 14 do 17%. Obje metode daju sličnu ponovljivost i točnost rezultata, ali je znatna razlika u njihovoj osjetljivosti.

Ključne riječi:
elektrotermalna atomskoapsorpcijska spektrometrija, granica detekcije, hidridna tehnika, ponovljivost, standardni referentni materijali, točnost mjerenja

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