

# Optimization of Selenium Determination in Vegetables, Fruits, and Dairy Products by Flow Injection Hydride Generation Atomic Absorption Spectrometry\*

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A flow injection hydride generation atomic absorption spectrometric (FI-HG-AAS) method was developed for determination of selenium in vegetables, fruits, and dairy products after microwave digestion of samples. The experimental conditions for FIAS 400 (concentration of reducing agent and carrier acid, time of reduction, flow rate of argon carrier gas) were optimized. The linearity range under optimized conditions was 0.20–25.0  $\mu\text{g dm}^{-3}$ .

The detection limit of the proposed method was 0.06  $\mu\text{g kg}^{-1}$  and limit of quantitation was 0.20  $\mu\text{g kg}^{-1}$ . The procedure was validated by the method of standard additions (5.0  $\mu\text{g dm}^{-3}$  and 10.0  $\mu\text{g dm}^{-3}$  in vegetables, fruits, and dairy products) and the recoveries were from 88 % to 104 %. The accuracy was evaluated using BCR 150 skin milk reference material (found: 0.128  $\text{mg kg}^{-1}$ ,  $s_x = 0.004 \text{ mg kg}^{-1}$ , certified: 0.132  $\text{mg kg}^{-1}$ ,  $s_x = 0.010 \text{ mg kg}^{-1}$ ). The combined standard uncertainty of selenium was 8.4 %. The method was developed and used for determination of selenium in vegetables, fruits, and dairy products consumed in Slovakia.

The trace mineral selenium is an essential nutrient of fundamental importance to human biology, because Se is a component of glutathione peroxidase. Some epidemiological studies have revealed an inverse correlation between Se status and cardiovascular disease [1]. Selenium deficiency has been suggested to have a role in the etiology of other pathologies, such as oxidative stress or inflammatory conditions, diabetes mellitus, hepatopathies, HIV infection [2–4]. The flow injection hydride generation technique combined with atomic absorption spectrometry (FI-HG-AAS) offers the advantage of good detectability, together with reduction of matrix interferences. Determination of selenium content in food by this technique also requires destruction of the organic matter and complete reduction of Se(VI) to Se(IV) [5–7]. Moreover, the method has to be optimized for each type of sample.

## EXPERIMENTAL

Fruits, vegetables samples, and dairy products were obtained from market network in Bratislava. Samples were digested in consumption form by microwave digestion system – Milestone MLS 1200 MEGA (Sorisole, Italy).

Standard reference material – selenium stock solution, 1  $\text{g dm}^{-3}$  in 2 %  $\text{HNO}_3$  (Slovak Institute of

Metrology, Bratislava, SR).

Certified reference material – BCR 150 skin milk (Se = 0.132  $\text{mg kg}^{-1}$ ,  $s_x = 0.010 \text{ mg kg}^{-1}$ ).

All reagents were of anal. grade: Nitric acid – 65 %, Suprapur (Merck), hydrochloric acid – 37 %, Suprapur (Merck), sodium tetrahydroborate – 99 % (Merck), sodium hydroxide p. a. (Lachema, Czech Republic).

Volatile selenium was generated in FIAS 400 flow injection analysis system, transported by means of an argon carrier gas to a quartz cell of the Perkin–Elmer 4100 atomic absorption spectrometer (Norwalk, CT, USA). In the quartz cell heated to 900 °C the hydrides were converted to gaseous metal atoms and the amount of light absorbed at  $\lambda = 196.0 \text{ nm}$  was measured. An electrodeless discharge lamp of Se operated at 210 mA and the external modulation from the EDL System 2 power supply was used.

## Digestion

Weighted 1.0–2.0 g of vegetable or fruit (0.5 g of dairy products) were introduced into a teflon microwave digestion vessel, and 2  $\text{cm}^3$  of  $\text{HNO}_3$  and 2  $\text{cm}^3$  of  $\text{HCl}$  (3  $\text{cm}^3$  of  $\text{HNO}_3$  and 1  $\text{cm}^3$  of  $\text{HCl}$ ) were added. The vessel was closed and fastened into the rotor. The rotor with 6 loaded vessels was placed into the microwave oven. The microwave digestion program

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was applied: 250 W (1 min), 0 W (1 min), 250 W (5 min), 400 W (5 min), and 650 W (5 min). The digested samples were adjusted to 10 cm<sup>3</sup> with HCl (1:1) and heating to 90°C. Temperature was maintained at 90°C for 30 min to ensure reduction of Se(VI) to Se(IV). Selenium was determined by FI-HG-AAS with optimized parameters. The peak areas of the absorbance were used for calculation of selenium content.

## RESULTS AND DISCUSSION

Parameters for determining Se were optimized: gas (argon) flow rate, contents of sodium tetrahydroborate used as reductant, HCl used as carrier solution, and time of reduction. These parameters were optimized with sample solution of the following matrix at a content of approximately 1 µg dm<sup>-3</sup> Se stock solution. Results of parameter optimization are presented in Tables 1—4. The data in the tables are mean values from three determinations with relative standard deviation from 3 % to 5 %. Recovery was assessed in vegetables, fruits, and dairy products by analysis of three fortified samples. Fortification of samples was performed before microwave digestion using solution of Se(VI). Selenium recoveries ranged from 88 % to 104 % are presented in Tables 5—7. Found selenium is the dif-

**Table 1.** Optimization of HCl Content Used as Carrier Solution

<i>w</i> (HCl)/%	1.5	3.0	6.0	9.0	12.0	15.0
Signal [AA] <sub>area</sub>	0.161	0.174	0.177	0.167	0.158	0.156
Signal [AA] <sub>hight</sub>	0.035	0.036	0.034	0.034	0.029	0.029

**Table 2.** Optimization of the Argon Flow Rate for FIAS Determination of Selenium

	Argon flow rates/(cm <sup>3</sup> min <sup>-1</sup> )				
	40	60	75	100	140
Signal [AA] <sub>area</sub>	0.200	0.200	0.198	0.210	0.158
Signal [AA] <sub>hight</sub>	0.039	0.044	0.043	0.044	0.029

**Table 3.** Signals [AA]<sub>area</sub> at the Optimization of NaBH<sub>4</sub> Content in 0.05 % NaOH as Reductant on Double Concentration Levels

$\rho$ (Se stock solution)/(µg dm <sup>-3</sup> )	<i>w</i> (NaBH <sub>4</sub> )/%			
	0.05	0.1	0.2	0.5
2.5	0.290	0.324	0.415	0.293
25.0	2.253	1.929	1.573	1.227

**Table 4.** Optimization of Time Reduction of Se(VI) into Se(IV)

Reduction time/min	15	30	45	60
Signal [AA] <sub>area</sub>	0.047	0.095	0.084	0.077
$\rho$ (µg dm <sup>-3</sup> )	0.49	0.99	0.87	0.80

**Table 5.** Recovery for Determination of Selenium by the FI-HG-AAS Method in Vegetables

$\rho$ (Se added)	$\rho$ (Se determined)	$\rho$ (Se found)	Recovery
µg dm <sup>-3</sup>	µg dm <sup>-3</sup>	µg dm <sup>-3</sup>	%
0	0.6	0	—
10	11	10.4	104.0
20	21	20.4	102.0

**Table 6.** Recovery for Determination of Selenium by the FI-HG-AAS Method in Fruits

$\rho$ (Se added)	$\rho$ (Se determined)	$\rho$ (Se found)	Recovery
µg dm <sup>-3</sup>	µg dm <sup>-3</sup>	µg dm <sup>-3</sup>	%
0	1.2	0	—
10	11.1	9.9	99.0
20	18.8	17.6	88.2

**Table 7.** Recovery for Determination of Selenium by the FI-HG-AAS Method in Dairy Products

$\rho$ (Se added)	$\rho$ (Se determined)	$\rho$ (Se found)	Recovery
µg dm <sup>-3</sup>	µg dm <sup>-3</sup>	µg dm <sup>-3</sup>	%
0	8.8	0	—
5	13.8	5	100.0
10	18.6	9.8	98.0

ference between selenium determined in the sample with selenium addition and selenium determined in the sample without selenium addition.

Optimized value of carrier solution is 3 % HCl and of the argon flow rate for FIAS determination of selenium 60—100 cm<sup>3</sup> min<sup>-1</sup>. Optimized content of NaBH<sub>4</sub> in 0.05 % NaOH aqueous solution as a reductant is 0.05 % and time of reduction of Se(VI) to Se(IV) is 30 min. This optimized method was used on measurement of selenium in vegetables, fruits, and dairy products (Table 8). Low level of selenium in vegetables (0.001—0.102 mg kg<sup>-1</sup>), fruits (0.002—0.1 mg kg<sup>-1</sup>), and dairy products (0.006—0.176 mg kg<sup>-1</sup>) was observed.

**Table 8.** Content of Selenium in Fruits, Vegetables, and Dairy Products

Sample	<i>n</i>	Minimum mg kg <sup>-1</sup>	Maximum mg kg <sup>-1</sup>	Average mg kg <sup>-1</sup>
Apricot	3	0.005	0.024	0.014
Apples	5	0.003	0.111	0.040
Plums	3	0.016	0.111	0.064
Pears	5	0.037	0.158	0.077
Grape	3	0.008	0.064	0.036
Grape-fruit	3	0.004	0.008	0.005
Banana	3	0.002	0.018	0.009
Pepper	5	0.006	0.102	0.050
Tomato	5	0.015	0.076	0.051
Carrot	3	0.001	0.015	0.007
Cucumber	3	0.007	0.030	0.015
Kohlrabi	2	0.030	0.045	–
Cabbage	2	0.041	0.064	–
Cauliflower	3	0.017	0.070	0.043
Milk	3	0.007	0.009	0.008
Cream	3	0.025	0.069	0.049
Yoghurt	3	0.006	0.119	0.044
Acid milk	3	0.024	0.075	0.041
Cheese	5	0.010	0.176	0.072

The detection limit defined as the selenium concentration of the reagent blanks corresponding to the three-fold standard deviation of these blanks ( $n = 10$ ), was  $0.06 \mu\text{g kg}^{-1}$ . Similarly, the quantitation limit corresponded to the ten-fold standard deviation of the reagent blanks and it was  $0.20 \mu\text{g kg}^{-1}$ . The trueness of the method was tested by determination of selenium concentration in BCR 150 skin milk reference material. The analyzed value was  $0.128 \text{ mg kg}^{-1}$ ,  $s_x$

$= 0.004 \text{ mg kg}^{-1}$ , while the certified value was  $0.132 \text{ mg kg}^{-1}$ ,  $s_x = 0.010 \text{ mg kg}^{-1}$ . The combined standard uncertainty  $u_C$  of selenium was 8.40 %, the A type uncertainty  $u_A$  was 3.30 % and B type uncertainty  $u_B$  7.76 %. The type B uncertainty includes a sample mass, volume, slope of calibration curve, and signal measured. The linearity range under optimized conditions was  $0.2\text{--}25.0 \mu\text{g dm}^{-3}$ .

## CONCLUSION

The presented optimized method is more economic than the routine analysis recommended by the FIAS system producer, because of the use of lower reductant and carrier solution concentration as well as lower carrier gas flow rate. The method was implemented for determination of selenium in vegetables, fruits, and dairy products consumed in Slovakia.

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