

Speciation of selenium and tellurium in milk by hydride generation atomic fluorescence spectrometry

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A simple, fast and highly sensitive method has been developed for the differentiation of Se and Te into their (iv) and (vi) oxidation states in milk samples by HG-AFS. This procedure involves a previous leaching of milk slurries by sonication with *aqua regia* for 10 min. Se(iv) and Te(iv) were determined by analysis of the samples without a pre-reduction step; being total Se and Te determined after reduction with KBr. The method provides limit of detection values (LOD) of 0.012 and 0.023 ng ml⁻¹ for Se(iv) and Te(iv) respectively. Average relative standard deviation values of 10.5%, 3.9%, 12% and 12.5% were found for the determination of Se(iv), Te(iv), Se(vi) and Te(vi) in milk samples containing Se from 11.1 to 32.2 ng g⁻¹ and Te from 1.1 to 10 ng g⁻¹. Recovery experiments made on actual samples spiked with Se(iv), Se(vi), Te(iv) and Te(vi) evidenced that the sonication with *aqua regia* does not modify the oxidation state of the elements considered.

Introduction

Speciation of trace elements is one of the last frontiers in elemental analysis,¹ it having been established that the pathways of trace elements depend on their specific chemical forms and oxidation states.² Milk is an important source of micronutrients, like selenium, and non-essential toxic elements, like tellurium. So, speciation studies on milk have an increasing relevance in nutritional and toxicological studies.³

There are several studies published about the total content of Se in milk samples^{4–10} but a single precedent on a methodology suitable for the determination of total Te in milk.¹⁰

Size exclusion chromatography coupled with ICP-MS¹¹ and capillary electrophoresis ICP-MS^{12,13} have been employed to evaluate the presence of Se species in human milk and infant formula. However, results reported are far from providing a simple methodology for the quantification of the different oxidation states of Se in natural samples.

Non-chromatographic methods of speciation¹⁴ offer an inexpensive and sensitive alternative for the fast determination of different oxidation states in real analysis. Based on this principle, Bryce and co-workers¹⁵ proposed the use of cathodic stripping voltammetry for the speciation of Se(iv) and Se(vi).

The main objective of our work is the development of a procedure for the AFS screening analysis of Se(iv) and Te(iv) directly on real samples.

Experimental

Apparatus and reagents

A continuous flow hydride generation atomic fluorescence spectrometer PSA Millennium Excalibur 10055 from PS Analytical (Orpington, UK), equipped with boosted discharge hollow cathode lamps for Se and Te from Photron (Victoria, Australia), was employed for hydride generation atomic fluorescence measurements (HG-AFS). An inductively coupled plasma mass spectrometer ICP-MS PerkinElmer Elan-5000 SCIEX (Thornhill, Ontario, Canada), equipped with a cross-flow nebulizer and an autosampler from PerkinElmer (Norwalk, USA), was used for reference measurements of total Se and Te in milk. A domestic microwave oven LG Intellwave

(Manchester, UK) of 2450 MHz frequency and operating at a maximum exit power of 700 W was employed for microwave-assisted digestion using hermetically sealed home made 120 ml internal volume PTFE reactors.

All reagents used were of the highest purity and all solutions were prepared in ultrapure water (resistivity of 18.2 MΩ cm) obtained from a Milli-Q system (Millipore, Bedford, USA).

Se(iv) stock solution was prepared from Na₂SeO₃·5H₂O by Fluka (Buchs, Switzerland) and Te(iv) standard stock solution was prepared from Na₂TeO₃ by Aldrich Chemicals (Milwaukee, WI). Se(vi) and Te(vi) standards were prepared by dissolving Na₂SeO₄ and H₂TeO₄ from Fluka. *Aqua regia* was prepared by mixing HNO₃ and HCl 1:3 from the concentrated suprapure solutions. Antifoam A from Sigma (Steinheim, Germany) was used to avoid foam formation during hydride generation. Reductant solution containing KBr 10% (m/v) was prepared from the corresponding salt from Merck (Darmstadt, Germany).

For ICP-MS measurements a multielemental standard solution containing 10 mg l⁻¹ Rh, Be, Ge, Re and Tb was prepared by dilution with 2% (v/v) HNO₃ and used for calibration by adding 0.1 ml of this solution to 10 ml of previously digested and diluted milk samples.

Experimental procedure

Slurries treatment for speciation of Se and Te using HG-AFS

2.0000 ± 0.0001 g milk were accurately weighted, 4 ml *aqua regia* and 2 ml antifoam A were added before dilution with 5 ml of water. The obtained slurry was sonicated for 10 min in an ultrasound water bath, 16 ml conc. HCl were added to the yellowish slurry and it was then diluted to 50 ml with water. A 2 ml aliquot of these samples was injected into the AFS system. The corresponding hydrides of Se(iv) and Te(iv) were formed by reaction with NaBH₄ and AFS measurements of samples were interpolated in the corresponding external calibration lines obtained from hydrochloric solutions of Se(iv) and Te(iv).

Measurements were also made after adding 10 ml of KBr 10% (m/v) as reductant to the milk slurries treated with *aqua regia*, diluted to 50 ml and heated at 60°C for 30 min in order to

Table 1 Experimental conditions for determination of Se and Te

HG-AFS measurements			ICP-MS determination after MW-assisted digestion	
Parameter	Selenium	Tellurium	Parameter	Operating conditions
Wavelength/nm	196.0	214.3	Rf power/W	1100
Primary current/mA	20	15	Sample uptake/ml min ⁻¹	1.0
Boost current/mA	25	17.5	Plasma Ar flow/l min ⁻¹	12
Sample volume/ml	2	2	Nebulizer Ar flow/l min ⁻¹	0.8
Delay time/s	10	10	Auxiliary Ar flow/l min ⁻¹	0.7
Analysis time/s	30	30	Vacuum pressure/mm Hg	9.6 × 10 ⁻⁶
Memory time/s	30	30	Dwell time/ms	250
Filter	32	45	Sweeps per reading	5
Measurement mode	Peak height	Peak height	Readings per replicate	1
HCl/mol l ⁻¹	4.5	4.5	Number of replicates	1
NaBH ₄ (%) (m/v)	1.2	1.2		
Ar flow rate/ml min ⁻¹	345	270		
Air flow rate/l min ⁻¹	2.5	2.5		
Blank flow rate/ml min ⁻¹	9	9		
Sample flow rate/ml min ⁻¹	9	9		
NaBH ₄ flow rate/ml min ⁻¹	4.5	4.5		
Reaction coil length/cm	150	150		

establish the total content of Se and Te by HG-AFS measurements after reduction of Se(vi) and Te(vi).

All measurements by HG-AFS were made in the instrumental conditions shown in Table 1.

Microwave-assisted digestion for total Se and Te determination by ICP-MS

2.0000 ± 0.0001 g milk were accurately weighted, introduced inside a PTFE reactor and evaporated to the dryness in an oven at 160 °C for 2 h. 1.5 ml conc. HNO₃ were added and the reactor placed inside a microwave oven. Samples were irradiated for 4 cycles of 1 min at 500 W, left to cool and 1.5 ml H₂O₂ 30% (m/v) and 0.5 ml conc. HNO₃ were added before irradiation for 4 additional cycles of 1 min at 500 W. At the end of this process completely clear solutions were obtained. Digested samples were transferred quantitatively to a 10 ml volumetric flask with HNO₃ 2% (v/v). 0.1 ml of a 10 mg l⁻¹ Rh, Be, Ge, Re and Tb solution in HNO₃ 2% (v/v) was added to the 10 ml volume of digested milk samples and measurements at the ⁸⁰Se and ¹²⁸Te mass numbers were made using the relative signals to Rh in the semiquantitative mode and employing the operation conditions indicated in Table 1 based on our previous experience working with the ICP-MS in the semi-quantitative mode.¹⁶

Results and discussion

Fast methodologies for HG-AFS analysis

Atomic fluorescence spectrometry offers a very sensitive and selective method for the determination of Se and Te. However, milk is a complex biological matrix that can not be directly fed up to the HG-AFS system due to the precipitation of proteins during the sample acidification which can block the reduction coils.

Previous studies on the direct determination of Se and Te in

milk samples which were deproteinated with HCl and filtered, provided non-quantitative results.¹⁰ Therefore the milk was treated with mixtures of HNO₃ and HCl and sonicated allowing the obtained slurries to be analysed without previous phase separation. It was confirmed, for As and Sb analysis,¹⁷ that inorganic compounds could be recovered quantitatively after 10 min sonication of 2 g samples with 4 ml *aqua regia*, it is necessary to reduce the slurries with KB for the determination of total Se and Te.¹⁸

A two step speciation analysis

Se(vi) and Te(vi) do not react with NaBH₄ to form the corresponding hydrides. Speciation of Se(iv), Se(vi) and Te(iv), Te(vi) can be easily carried out by hydride generation atomic fluorescence using a two step analysis. Samples must be analysed without pre-reduction, in order to obtain Se(iv) and Te(iv) concentrations; then, the sample can be treated with KBr in acidic medium at 60 °C for 30 min in order to achieve a quantitative reduction of any Se(vi) and Te(vi) present in the sample. The analysis of this last solution gives total concentration of selenium and tellurium. Se(vi) and Te(vi) can be calculated by the difference between the two analysis performed.

Analytical figures of merit

Table 2 summarizes the typical calibration lines obtained for the determination of Se(iv) and Te(iv) by HG-AFS and the main figures of merit for the determination of these species in addition to Se(vi) and Te(vi).

The limit of detection values were established for a probability level of 99.6% (*k* = 3) and were 0.012 ng ml⁻¹ for Se and 0.023 ng ml⁻¹ for Te. The average relative standard deviation of three replicate analysis of 10 different commercially available samples (see Table 2), were 10.5% for Se(iv), 3.9% for Se(vi), 12% for Te(iv) and 12.5% for Te(vi). An

Table 2 Analytical figures of merit of the HG-AFS developed method for the determination of Se and Te inorganic species in milk

Element	LOD/ng ml ⁻¹	LOD/ng g ⁻¹	Calibration equation ^a	R ²	RSD (%) ^b	RSD (%) ^c
Se(iv)	0.012	0.30	(1330 ± 20)x + (40 ± 11)	0.9994	10.5%	2.5%
Se(vi)					3.9%	
Te(iv)	0.023	0.57	(720 ± 45)x + (18 ± 12)	0.9966	12%	6.1%
Te(vi)					12.5%	

^a A minimum of five standards and a blank were used for each calibration line in the concentration range from 0 to 2 ng ml⁻¹. ^b The mean relative standard deviation was established from the average of the variation coefficient found for three replicate analysis of ten commercially available samples with different contents of Se(iv), Se(vi), Te(iv) and Te(vi). ^c The relative standard deviation was established from five independent measurements of solutions containing 1 ng ml⁻¹ of each one of the elements considered.

Table 3 Analysis of Se and Te species in commercially available milk samples made by HG-AFS using milk slurries sonicated with *aqua regia*

Sample	Se(IV)/ng g ⁻¹	Se(VI)/ng g ⁻¹	Sum/ng g ⁻¹	Se total ^a	Te(IV)/ng g ⁻¹	Te(VI)/ng g ⁻¹	Sum/ng g ⁻¹	Te total ^a
1 Full cream	4.1 ± 0.4	8.4 ± 0.6	12.5 ± 0.7	12.5 ± 0.4	3.9 ± 0.3	2.3 ± 0.1	6.2 ± 0.3	6.1 ± 0.2
2 Skimmed	4.2 ± 0.6	6.9 ± 0.4	11.1 ± 0.7	11.1 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	1.1 ± 0.2	1.04 ± 0.17
3 Full cream with Ca	3.9 ± 0.7	11.6 ± 0.4	15.5 ± 0.8	15.4 ± 0.5	3.9 ± 0.3	3.8 ± 0.1	8.6 ± 0.3	8.5 ± 0.3
4 Partially-skimmed	4.9 ± 0.6	9.6 ± 0.7	14.5 ± 0.9	14.4 ± 0.6	4.8 ± 0.7	2.3 ± 0.2	6.2 ± 0.7	6.2 ± 0.9
5 Skimmed with vegetable fat	3.2 ± 0.4	11.0 ± 0.3	14.2 ± 0.5	14.2 ± 0.6	1.9 ± 0.5	0.9 ± 0.3	2.8 ± 0.6	2.8 ± 0.9
6 Skimmed with folic acid	4.2 ± 0.4	19.1 ± 0.5	23.3 ± 0.6	23.3 ± 0.8	5.4 ± 0.5	3.5 ± 0.3	8.9 ± 0.6	9.1 ± 0.3
7 Skimmed Omega 3	5.5 ± 0.4	15.7 ± 0.3	21.2 ± 0.5	21.3 ± 0.7	6.2 ± 0.5	3.6 ± 0.5	9.8 ± 0.7	9.7 ± 0.5
8 Partially-skimmed with propolis	5.6 ± 0.6	20.7 ± 0.4	26.3 ± 0.7	26.0 ± 0.3	5.4 ± 0.4	3.6 ± 0.4	9.0 ± 0.6	9 ± 1
9 Partially-skimmed with vitamins and minerals	5.3 ± 0.4	17.1 ± 0.5	22.4 ± 0.6	22.3 ± 0.2	6.3 ± 0.2	3.2 ± 0.4	9.5 ± 0.4	9.3 ± 0.5
10 Goat milk	9.8 ± 0.3	22.4 ± 0.6	32.2 ± 0.7	33.6 ± 0.4	5.8 ± 0.4	4.2 ± 0.2	10 ± 0.4	10.1 ± 0.3

^a Total Se and Te determined after microwave-assisted digestion HG-AFS.**Table 4** Recovery of Se and Te species from spiked milk samples using HG-AFS of sample slurries sonicated with *aqua regia*

Sample	Se(IV) added/ ng g ⁻¹	Se(VI) added/ ng g ⁻¹	Recovery Se(IV) (%)	Recovery Se(VI) (%)	Te(IV) added/ ng g ⁻¹	Te(VI) added/ ng g ⁻¹	Recovery Te(IV) (%)	Recovery Te(VI) (%)
1a Full cream	5	0	101.1	—	5	0	99.6	—
1b	0	5	—	97.9	0	5	—	96.2
1c	2.5	2.5	102.7	98.4	2.5	2.5	97.8	94.1
2a Skimmed	4	0	98.4	—	1	0	104.4	—
2b	0	4	—	101.7	0	1	—	94.0
2c	2	2	100.6	100.8	0.5	0.5	101.1	102.3
4a Partially-skimmed	3	0	98.3	—	2	0	95.8	—
4b	0	3	—	96.2	0	2	—	98.1
4c	1.5	1.5	93.5	102.2	1	1	93.7	96.4
6a Skimmed with folic acid	2	0	100.4	—	3	0	95.6	—
6b	0	2	—	102.2	0	3	—	97.2
6c	1	1	99.9	104.6	1.5	1.5	92.1	94.1
8a Partially-skimmed with propolis	1	0	101.7	—	4	0	107.7	—
8b	0	1	—	101.4	0	4	—	99.6
8c	0.5	0.5	103.5	104.5	2	2	96.6	93.5
9a Partially-skimmed with vitamins and minerals	2	0	99.1	—	2	0	104.8	—
9b	0	2	—	103.6	0	2	—	93.7
9c	1	1	101.5	104.9	1	1	97.2	92.7

additional evaluation of the precision of the method was established for the repeatability of five independent determinations of a solution containing 1 ng ml⁻¹ of Se(IV) and Te(IV) and relative standard deviation values of 2.5% and 6.1% were obtained respectively.

Analysis of milk samples

Milk samples were selected from different parts of Spain and with different industrial processes, including: (1) full cream, (2) skimmed, (3) full cream with Ca, (4) partially skimmed, (5) skimmed with vegetable fat, (6) skimmed with folic acid, (7) skimmed with omega 3 acid, (8) partially skimmed with propolis, (9) partially skimmed with vitamins and minerals, all those corresponding to cow milk and (10) a goat milk.

Results found by the developed methodology for Se and Te species are shown in Table 3. Total Se and Te were also determined by HG-AFS after microwave-assisted digestion.

Se(VI) appears to constitute a predominant species in milk, which varies from 6.9 ng g⁻¹ (for skimmed cow milk) to 22.4 ng g⁻¹ (for goat milk). The regression between the sum of Se(IV) and Se(VI) concentration obtained and total Se found after microwave-assisted digestion provided an equation $y = (0.96 \pm 0.02)x + (0.7 \pm 0.4)$ with a regression coefficient $R = 0.98$, being experimental t values for the intercept and the slope of 1.84 and 2.12, which are lower than the theoretical t value (2.23) corresponding to a 95% probability and 10 freedom degrees, thus evidencing that the sum of the two oxidation states found for Se in milk by the proposed methodology is statistically comparable with the total content of this element determined after complete sample digestion.

Te(IV) and Te(VI) species are in a nearly 6:4 proportion; Te(IV) varies from 0.7 to 6.3 ng g⁻¹ and Te(VI) from 0.4 to 4.2 ng g⁻¹. Total Te found after microwave-assisted digestion compares well with the sum of Te(VI) and Te(IV), giving the regression equation $y = (0.996 \pm 0.013)x + (0.1 \pm 0.1)$ with a regression

Table 5 Comparison between data found for speciation analysis of Se and Te in milk samples by HG-AFS and those obtained for total Se and Te by ICP-MS after microwave-assisted digestion

Sample	HG-AFS		ICP-MS	
	Sum of Se species	Sum of Te species	Total Se	Total Te
1 Full cream	12.5 ± 0.7	6.2 ± 0.3	13.0 ± 0.4	5.7 ± 0.5
2 Skimmed	11.1 ± 0.7	1.1 ± 0.2	11.5 ± 0.3	0.6 ± 0.4
3 Full cream with Ca	15.5 ± 0.8	8.6 ± 0.3	14.4 ± 0.7	8.1 ± 0.3
4 Partially-skimmed	14.5 ± 0.9	6.2 ± 0.7	15.2 ± 0.6	5.6 ± 0.8
8 Partially-skimmed with propolis	26.3 ± 0.7	9.0 ± 0.6	24.6 ± 0.8	7.6 ± 0.7

coefficient $R = 0.99$. Experimental t values for the intercept and the slope were 0.54 and 0.31, lower than the theoretical value (2.23). It can therefore be concluded that sonication of milk samples with *aqua regia* for 10 min is enough to quantitatively extract Te. A previous reduction of the slurry with KBr being necessary for total HG-AFS determination because part of the Te present in the original sample remains in the Te(vi) state. So, in general it can be confirmed that the methodology developed in the present study can differentiate samples from different origins and industrial processes.

Method validation

In order to evaluate the preservation of the original oxidation state of the two elements under study, six samples were spiked with variable amounts of Se(IV), Se(VI), Te(IV) and Te(VI) concentrations, in all cases at concentration levels lower than those found in actual samples (see Table 4).

Recovery values varied from 93.5 to 103.5% for Se(IV), from 96.2 to 104.9% for Se(VI), from 92.1 to 104.8% for Te(IV) and from 92.7 to 102.3% for Te(VI), thus evidencing that there is no alteration or losses of the Se and Te species during sample sonication with *aqua regia*.

Further validation of the developed method was carried out by comparison of the sum of data found by speciation of Se and Te in sonicated samples by HG-AFS with those obtained by ICP-MS after microwave-assisted digestion of samples (Table 5).

There is an evident correlation between data found for the sum of Se(IV) and Se(VI) determined by slurries (y) and data obtained by ICP-MS (x), giving the regression equation $y = (0.97 \pm 0.03)x + (0.7 \pm 0.5)$ and the regression coefficient $R = 0.98$.

On the other hand, regression between the sum of Te(IV) and Te(VI) (y) and data found after microwave-assisted ICP-MS analysis (x) was $y = (1.0 \pm 0.1)x - (0.4 \pm 0.5)$ $R = 0.95$. So, it can be concluded that both data populations are coincident.

Statistical t values found for the intercept and slope were 1.01 and 1.41 for Se, and 0.17 and 0.99 for Te, being lower than the theoretical t values, 1.77, found for a 95% probability level and 13 freedom degrees.

Online waste treatment

The liquid waste obtained after HG-AFS measurements was merged on-line with a 4 ml min^{-1} flow of a 20 mg l^{-1} Fe(III) solution and with a 4 ml min^{-1} flow of a 3.5 mol l^{-1} NaOH solution in order to neutralize the waste and to remove toxic

elements through coprecipitation with Fe(OH)_3 . Based on this approach 1000 ml liquid waste were reduced to approximately 5 g solid passivated residue, contributing to the elimination of side effects of the analytical methodology.

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