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Food Chemistry 91 (2005) 181–189

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Multicommutation hydride generation atomic fluorescence determination of inorganic tellurium species in milk

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Received 3 May 2004; received in revised form 8 July 2004; accepted 12 August 2004

Abstract

A multicommutated flow system has been developed for hydride generation, atomic fluorescence (HG-AFS) determination of tellurate (Te^{VI}) and tellurite (Te^{IV}) in milk samples. After a batch leaching of Te by sonication at room temperature for 10 min with aqua regia, sample slurries in acidic medium were merged with sodium borohydride and HCl to obtain data on Te^{IV}. Another portion of the acidic slurry was mixed with KBr and passed through a reaction coil introduced inside a microwave oven to reduce quantitatively Te^{VI} to Te^{IV} which was analyzed by HG-AFS. The detection limit was 0.57 $ng\,g^{-1}$ in the original samples. The linear range obtained was till 4 $ng\,ml^{-1}$ and the average recovery of different amounts of Te^{VI} and Te^{IV} added to real milk samples were 98 ± 4% and 98 ± 2%, respectively, indicating the absence of analyte losses or contaminations and original species modification. Average relative standard deviation of 6.3% was found for Te determination in a series of commercially available milk samples containing from 1.0 to 10.1 $ng\,ml^{-1}$ total Te. The proposed method provided a high sampling frequency of 24 h^{-1} for the determination of both, free Te^{IV} and total Te, in a same sample with a two times reduced waste generation and a four times reduced reagent consumption as compared with the continuous hydride generation. Additionally, the method developed requires a minimum operator attention and sample manipulation.

Keywords: Multicommutation; Speciation; Tellurium; On-line microwave-assisted reduction; Hydride generation atomic fluorescence spectrometry; Milk analysis

1. Introduction

Tellurium is a non-essential toxic element widely spread in nature, usually at low concentration levels (Klaasen & Watkins, 1999). However, tellurium could be accumulated in milk samples in which it replaces the essential selenium. Thus, it is important to determine the content of Te in milk. Furthermore, the fact that the toxicity of Te depends on its oxidation state, tellurite is 10 times more toxic than tellurate, is an important reason to look for suitable methodologies in order to provide information about inorganic Te species in food, as well as to obtain the total Te content (Yu, Cai, Guo, Yang, & Khoo, 2003).

Hydride generation coupled with atomic fluorescence spectrometry HG-AFS is one of the most powerful analytical tools for ultra trace determination of heavy elements belonging to IVa–VIa groups, such as tellurium. HG has a marked selectivity for Te^{IV} determination, since only this oxidation state is able to generate volatile species in diluted hydrochloric media.

In comparison with extensive speciation studies with other elements, such as arsenic and selenium, only few works have dealt with the speciation of tellurium. The methods proposed for Te speciation are complex and involve several sequential steps, thus making their use very difficult in routine analysis.

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doi:10.1016/j.foodchem.2004.08.016

Narukawa (1999) described a procedure for the fractionation and determination of Te^{IV} and Te^{VI} using a tungsten furnace and this procedure was based on the use of cobalt(III) oxide powder as collector and 3-phenyl-5-mercapto-1,3,4-thiadiazole-2(3H)-thione potassium salt (bismuthiol II) as auxiliary agent for the discrimination between Te^{IV} and Te^{VI} always depending on the pH of the sample solution.

Korez, Eroglu, Volkan, and Atamon (2000) suggested a separation–preconcentration method using a mercapto-modified silica microcolumn for the determination of trace amounts of Te^{IV} in waters by HG-AAS technique.

Yu et al. (2003) depicted the speciation analysis of tellurium by using solid-phase extraction in the presence of ammonium pyrrolidine dithiocarbamate and the detection was carried out by inductively coupled plasma mass spectrometry.

Recently, our research group has developed a highly sensitive non-chromatographic procedure (Cava-Montesinos, de la Guardia, Teustch, Cervera, & de la Guardia, 2004) for the speciation of Se and Te in milk samples basing our research on HG-AFS determination before and after the pre-reduction with solid KBr at 80 °C during 30 min inside a water bath.

On-line microwave-assisted procedures have received an increased attention as they are very fast and can be integrated in the fully mechanization of the complete analytical process, from sample pre-treatment to the final determination of the analytes (Carbonell, de la Guardia, Salvador, Burguera, & Burguera, 1990; Carbonell et al., 1992; de la Guardia, Carbonell, Morales-Rubio, & Salvador, 1993).

Furthermore, the potential of the multicommutation approach as a way for mechanization of AFS determinations has been demonstrated recently in both, the reduction of reagents consumption and the increase of the sampling throughput for the determination of Hg by cold vapour – AFS in water (Reis, Ródenas-Torralba, Sancenón-Buleo, Morales-Rubio, & de la Guardia, 2003) and milk (Cava-Montesinos, Ródenas-Torralba, Morales-Rubio, Cervera, & de la Guardia, 2004) and in the determination of Bi by HG-AFS in milk shake samples (Ventura-Gayete, Ródenas-Torralba, Morales-Rubio, Garrigues, & de la Guardia, 2004).

The use of microwave treatments integrated with multicommutation and flow injection has several goals such as the following: (i) to develop novel procedures for sample digestion, (ii) to reduce the time involved in the aforementioned operations, (iii) to increase the automation level of these steps, (iv) to simplify the processes, (v) to reduce the consumption of reagents in general, and (vi) to avoid the use of dangerous chemicals, in particular. These last two objectives do agree with the current green chemistry trends of minimizing contamination of the environment. The scientific community requires a dramatic reduction of the toxicity and volumes of chemicals emitted to the environment. Moreover, multicommutation manages the chemical reagents and implements different reaction conditions in order to determine the involved species without changing the flow manifold.

In the proposed methodology, the speciation of inorganic tellurium by multicommutation HG-AFS has been developed. The multicommutation approach was employed in order to implement all the necessary conditions for the speciation of tellurium with the proposed manifold. Thus, the main aim of this report was to research a pre-reduction of Te^{VI} to Te^{IV} by KBr in a hydrochloric acidic medium with the help of microwave radiation. The method was tested with different milk samples and compared to complete digestion procedures (Cava-Montesinos, Cervera, Pastor, & de la Guardia, 2003; Cava-Montesinos, Cervera, Pastor, & de la Guardia, 2004).

2. Experimental

2.1. Apparatus and flow set-up

The multicommutation flow network was assembled with a set of four three-way solenoid valves NResearch 161T031 (West Caldwell, USA). A PC 486 microcomputer with electronic interfaces Advantech Corp. PCL 711S controlled the commutation devices and performed data acquisition and processing. The software was written in QUICK BASIC 4.5. An electronic interface was used to generate an electric potential of 12 V and a 100 mA current required to switch the solenoid valves.

A Gilson Minipuls 3 peristaltic pump Model M312 (Villiers-le Bel, France) was employed to propel the reagent solutions and sample slurries.

Microwave digestion was performed with a domestic MW oven LG Intellowave (Manchester, UK) operating at the maximum exit power of 700 W. MW was equipped with a magnetron of 2450 MHz. On-line reduction of Te^{VI} to Te^{IV} was achieved in a 3 m length (0.8 mm i.d.) PTFE coil located inside the microwave oven.

For tellurium measurements, a HG-AFS PSA Millennium Excalibur 10055 from PS Analytical (Orpington, UK) was equipped with two independent peristaltic pumps a boosted discharge hollow cathode lamp for Te, from Photron (Victoria, Australia), and a specific filter was employed. The gaseous hydride formed, after mixing the sample with $NaBH_4$, was separated by a gas–liquid separator, passed through a hygroscopic membrane Perma Pure® (Farmingdale, NJ, USA) and atomised using a hydrogen diffusion mini-flame fed by the excess of H_2 generated by the reaction between HCl and $NaBH_4$. Mixing coils and transmission lines were made of polyethylene tubing (0.8 and 1.0 mm i.d.). Operating and chemical conditions employed by both, multicommutation and batch analysis, are given in Table 1.

The manifold employed for the mechanization of inorganic tellurium species determination is indicated in Fig. 1. It is integrated by two parts, the first one for on-line microwave-assisted pre-reduction of Te^{VI} to Te^{IV} and the second one for the hydride generation and AFS determination of free Te^{IV} directly from the sample slurries and total Te after their reduction.

Sample slurries, obtained by in batch sonication of milk with aqua regia, were sequentially aspirated on using valve V_2 for free Te^{IV} determination and by using valve V_1 for the on-line reduction of Te^{VI} to Te^{IV} with KBr and determination of total Te through aspiration using valve V_2.

Solenoid valves V_3 and V_4 were employed for the introduction of $NaBH_4$ and HCl prior to the HG-AFS determination of both, Te^{IV} and total Te. The pre-reduction manifold includes also a closed system for cool water recirculation, in order to avoid magnetron damage during sample treatment and an ice bath to cool the previously reduced sample slurry, being incorporated a well stirred open vessel to vent the samples before to be introduced in the HG system and to maintain stable the solid dispersion.

2.2. Reagents and samples

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

Stock solutions of 1000 $mg\,l^{-1}$ Te^{IV} and 1000 $mg\,l^{-1}$ Te^{VI} were prepared by dissolving the appropriate amounts of Na_2TeO_3 and H_6O_6Te, respectively, from Merck (Darmstadt, Germany) in ultrapure water. KBr, used as reductant agent, was obtained from Panreac (Barcelona, Spain). Sodium tetrahydroborate solutions were prepared from the solid product Fluka (Steinheim, Germany) dissolved in NaOH 0.1 $mol\,l^{-1}$ Probus (Barcelona, Spain). The $NaBH_4$ solutions were filtered through a 0.45 μm nylon membrane Lida (Kenosha, USA). Calibration and $NaBH_4$ solutions were prepared daily.

Table 1
HG-AFS operating parameters used for Te^{IV} and total inorganic Te determination in milk samples by both, multicommutation and batch modes

	Multicommutation	Batch
Atomic fluorescence spectrometer parameters		
Wavelength (nm)	214.3	
Measurement mode	Peak height	
Primary current (mA)	15	
Boost current (mA)	17.5	
Filter	45	
Multicommutation and continuous hydride generation parameters		
Gas–liquid separator (ml)	17	
Reaction coil length (cm)	–	150
B_1 coil length (cm)	300	–
B_2 coil length (cm)	200	–
B_3 coil length (cm)	47	–
ON/OFF sampling time (s) S_1 (ON)/S_1 (OFF)	0.25/0.25	–
ON/OFF reductant time (s)R_1 (ON)/R_1 (OFF)	0.25/0.25	–
ON/OFF sampling time (s) S_2 (ON)/S_2 (OFF)	0.75/0.25	–
Sampling time cycles	30	–
ON/OFF reductant time (s) R_2(ON)/R_2(OFF)	30/0	–
ON/OFF blank time (s) R_3(ON)/R_3(OFF)	0.25/0.75	–
Blank time cycles	30	–
Reagent concentrations		
KBr R_1 (%, w/v)	30	
$NaBH_4$ R_2 (%, w/v)	1.2	
HCl R_3 ($mol\,l^{-1}$)	4.0	
Sample and reagent flow rates		
KBr R_1 ($ml\,min^{-1}$)	4.6	
$NaBH_4$ R_2 ($ml\,min^{-1}$)	6.5	
HCl R_3 ($ml\,min^{-1}$)	9.3	
Sample S_1 ($ml\,min^{-1}$)	4.6	
Sample S_2 ($ml\,min^{-1}$)	8.5	
Ar ($ml\,min^{-1}$)	200	330
Air ($ml\,min^{-1}$)	250	250

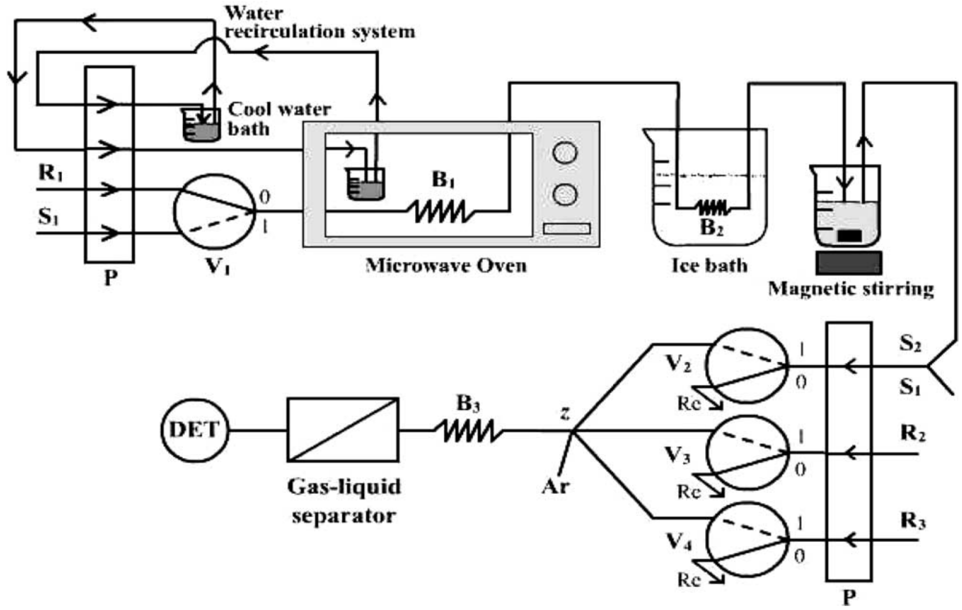

Fig. 1. Flow manifold for multicommutation HG-AFS speciation of inorganic tellurium. V_i: three-way solenoid valves (dashed lines represent the flow paths when the valves are in position 1); S_1: sample and standard solutions for total Te determination; S_2: sample and standard solutions for Te^{IV} determination; R_1: KBr reductant solution at flow rate of 4.6 ml min $^{-1}$; R_2: $NaBH_4$ reductant solution at flow rate of 6.5 ml min^{-1}; R_3: HCl blank solution at flow rate of 9.3 ml min^{-1}; B_1: reduction reaction coil, 3 m length; B_2: cooling coil, 2 m length; B_3: mixing coil, 47 cm length. Det: detector; Re: recycling to the corresponding vessels; P: peristaltic pumps; *z*: merging zone machined in acrylic.

Aqua regia was prepared by mixing HNO_3 and HCl 1:3 (v/v) from the concentrated suprapure solutions from Scharlau (Barcelona, Spain) and used for previous in batch leaching of Te from milk samples. Antifoam A, from Sigma (Steinheim, Germany), was used to stabilize the slurries and to avoid foam formation during hydride generation.

Synthetic air and high purity argon C-45 (99.995%), from *Carburos Metálicos* (Barcelona, Spain), were employed to dry volatile hydrides in the Perma Pure® membrane system and to transport them to the atomiser, respectively.

Ten commercial milk samples were analysed by using the developed procedure and data obtained were compared with those found by HG-AFS after complete digestion of samples by dry ashing (Cava-Montesinos et al., 2003) and by microwave assisted treatment in sealed reactors (Cava-Montesinos et al., 2004).

2.3. Multicommutation procedure

Two portions of 1.0000 ± 0.0001 g of milk were accurately weighed in 25 ml volumetric flasks, and 2 ml aqua regia and 1 ml antifoam A were added to each one before sonication in an ultrasound water bath for 10 min, following the treatment suggested in a previous study (Cava-Montesinos et al., 2004). After that, the obtained slurries were acidified with conc. HCl to a final concentration of 4.0 mol l^{-1} and diluted in both cases to 25 ml with water.

Using the manifold depicted in Fig. 1 the valves switching course shown in Table 2, the acidic sample slurries were aspirated alternately in the HG-AFS system using V_1 and V_2 or only V_2, depending of total Te or free Te^{IV} determination.

The sample slurry obtained after sonication was mixed with KBr for total Te determination. It was passed through the reaction coil located inside the MW oven by using valve V_1 (0.25/0.25 s) at a flow rate of 4.6 ml min^{-1}.

During Te^{VI} pre-reduction, in the B_1 coil, the microwave oven was operated at 700 W and valves V_2, V_3 and V_4 did not take part in the process. At the outside of the microwave oven, the treated slurry was cooled, in an ice bath, and vented and homogenized in an open beaker before feeding the pretreated samples into the HG-AFS system through valve V_2.

To determine free Te^{IV} and total Te the solenoid valve V_2 was switched to position 1 (0.75 s) and to position 0 (0.25 s) 30 cycles during the sampling time interval inserting sample solution S_1 directly, for free Te^{IV} determination, or pre-reduced slurry S_2 for total Te determination. During this time, a reductant solution (R_2) was also introduced through valve V_3 and both merged in the coil B_3, as can be seen in Fig. 1. At the same time, valve V_4 was switched to position 1 and 0, 0.25/0.75 s to insert into the coil B_3 a set of blank solution, as can be seen in step 2 shown in Table 2.

During the reading time, valves V_3 and V_4 were commutated. Valves inserting reductant solution (valve V_3)

Table 2
Valve switching course for the sequential determination of Te^{IV} and total Te by HG-AFS

Step	V_1	V_2	V_3	V_4	t (s)	Waste volume (μl)	Description of the task
1	1	–	–	–	0.25[a]	–	Microwave-assisted pre-reduction
	0	–	–	–	0.25		
2[b]	–	1	1	0	0.75	7600	Sample + reductant insertion
	–	0	1	1	0.25		Blank + reductant insertion
3[c]	–	0	0	1	1.0	3687	Transport of sample zone. Hydride separation step. Signal reading
	–	0	1	0	1.0		

0 and 1 represent that the solenoid valves are switched OFF and ON, respectively (see Fig. 1 for details). Determination of total Te: steps 1 to 3. Determination of Te^{IV}: steps 2 and 3. The waste volume was established from each sample or standard measurement.

[a] V_1 was switched in position 1 or 0 the necessary number of cycles to introduce KBr and sample solutions into B_1 reaction coil till to treat the original 25 ml sample slurry.

[b] This mixing time sequence was repeated 30 times, corresponding to 30 s.

[c] Step corresponding to the period defined as reading time, repeated seven times (14 s).

and HCl solution (valve V_4) were switched ON/OFF a set of times of 1.0/1.0 s during 14 s, as it is indicated in the step 3 of Table 2. The insertion of R_2 and R_3 solutions in the gas–liquid separation chamber is a necessary condition to maintain the mini-hydrogen diffusion flame burn up.

Data found from the fluorescence of samples were interpolated in a calibration line obtained from the insertion of Te^{IV} standards through valve V_2 and were used the same conditions than from samples.

3. Results and discussion

The determination of Te^{VI} and Te^{IV} in a conventional HG-AFS system is difficult because tellurate does not provide any fluorescence signal. Moreover, our preliminary studies based on the on-line reactions by using microwave-assisted treatments directly coupled to AFS measurements produced signal instability due to the overpressure generated during the on-line reduction of tellurate which extinguished the flame. These drawbacks were circumvented by using the set-up indicated in Fig. 1, exploiting the use of separate discrete commutation devices for a microwave-assisted reduction and fluorescence determination.

3.1. Determination of Te by using slurries

Previous studies made in our laboratory evidenced that the sonication at room temperature of 1 g milk with 2 ml aqua regia for 10 min was enough for the quantitative extraction of As, Sb, Se, Te and Bi (Cava-Montesinos et al., 2004). However, this treatment does not provide acidic slurries from which the corresponding hydride could be generated for total Te determination and a previous reduction of Te^{VI} to Te^{IV} was necessary.

3.2. Speciation analysis strategy

Te^{IV} can be directly determined by HG-AFS and speciation of inorganic forms, Te^{IV} and Te^{VI}, can be easily carried out by making measurements before and after the pre-reduction, in order to obtain Te^{IV} and total Te, respectively. Te^{VI} can be calculated by the difference between the two analysis performed.

KBr reductant solution has been extensively used to determine total Te, but the recommended concentrations of the reagents differ significantly (He, Moreda-Piñeiro, Cervera, & de la Guardia, 1998). Thus, the concentration of the reagent was varied in order to achieve the best sensitivity and precision.

The complete on-line reduction of Te^{VI} to Te^{IV} was assured by evaluating other parameters such as the reduction coil length and the carrier flow rates which control the residence time of the sample inside the microwave oven.

3.2.1. Reductant concentration effect on fluorescence signal

Concentrated HCl is usually employed for the reduction of tellurate to tellurite but KBr can be used to reduce the samples, thereby avoiding the deleterious effect of sample dilution involved in the reduction with HCl.

To ascertain the most appropriate KBr concentration, the fluorescence signals obtained for total Te in an actual full cream milk sample were evaluated by varying the reductant concentration from 20% to 50% (w/v) for different coil lengths of 2, 3 and 4 m, inside the oven operating at 700 W. As can be seen in Fig. 2, an increase in the KBr concentration increases also the reduction yield up to reach a maximum value, which depends on the reaction coil length. It seems clear that a KBr concentration of 30% (w/v) and a reaction coil of 3 m provide the most sensitive results. Hence, these conditions were chosen for further experiments.

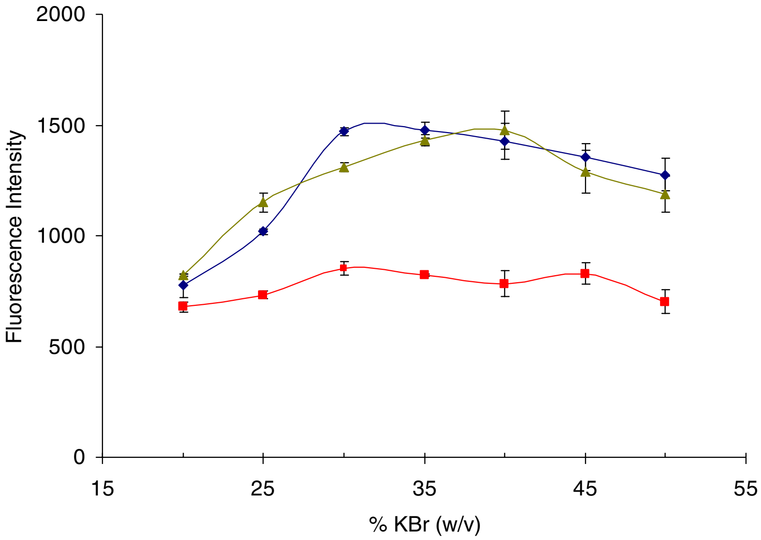

Fig. 2. Effect of KBr concentration on the fluorescence signal of total Te in a full cream milk with 6.1 ng ml^{-1} Te. In these experiments, the flow rate was fixed at 4.6 ml min^{-1}. Bars indicate the variability (±s) of three independent assays using different reduction coil lengths: (■) 2 m; (♦) 3 m; (▲) 4 m.

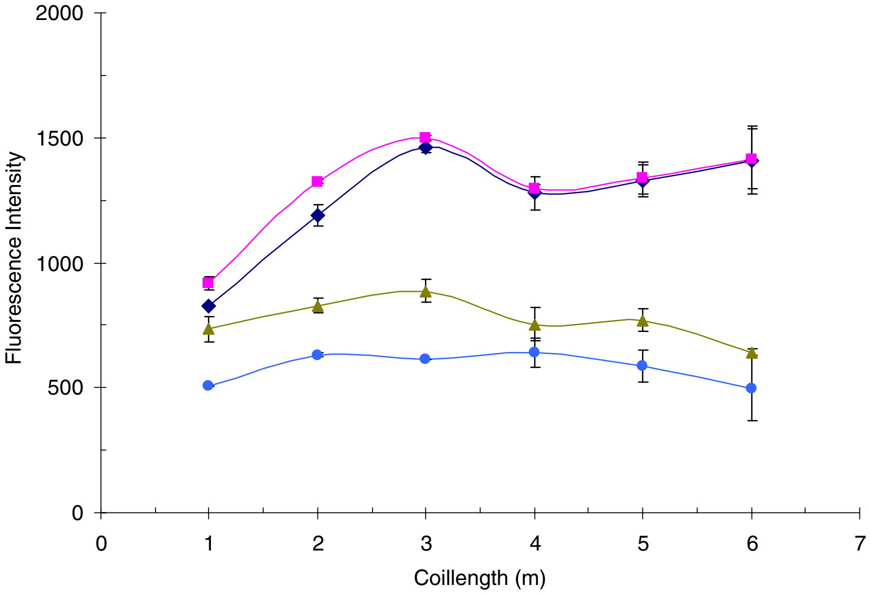

Fig. 3. Effect of the reduction coil length for different carrier flow rates on the fluorescence signals of a full cream cow milk with 6.1 ng ml^{-1} Te. In all experiences it was used a KBr concentration of 30% (w/v). Measurements were made using the multicommutation approach and error bars indicate the variability (±s) of three independent assays. (♦) 3.4 ml min^{-1}; (■) 4.6 ml min^{-1}; (▲) 5.7 ml min^{-1}; (●) 6.5 ml min^{-1}.

3.2.2. *Reduction coil length and carrier flow rate effects on fluorescence intensity*

Different coil length reactors were placed inside the microwave oven to reduce Te^{VI} to Te^{IV}. Fig. 3 shows the fluorescence signals obtained for a full cream sample treated with 30% (w/v) KBr and using a 700 W power radiation at different flow rates, from 3.4 to 6.5 ml min^{-1} and for different coil lengths, from 1 to 6 m.

As can be seen, the increase of the flow rate above 4.6 ml min^{-1} involves a drastic reduction of the fluorescence signals. It could be due to an incomplete reduction of Te^{VI} because of the small residence time of the sample inside the oven. For 3.4 and 4.6 ml min^{-1} flow rates, were found comparables results, increasing the fluorescence signals on increasing the reaction coil length, till to reach a maximum value for 3 m, from which the dispersion effects in the flow manifold tend to reduce the fluorescence signals and to increase the variability of the results. Thus, 4.6 ml min^{-1} and 3 m coil length seem to be the best compromise between the maximum reduction yield of Te^{VI} to Te^{IV} and the minimum dispersion, offering also a sampling throughput of 24 h^{-1}.

A 25 ml volume of a milk sample slurry could be treated for microwave-assisted reduction of Te^{VI} to Te^{IV} in approximately 326 s before aspirating a discrete volume of this reduce slurry into the multicommutation HG-AFS system to do the determination of total Te. It provides a dramatic decrease of the time required for the reduction of Te^{VI} as compared with the approximately 30 min involved in the batch reaction (Ródenas-Torralba, Cava-Montesinos, Morales-Rubio, Cervera, & de la Guardia, 2004).

3.3. Analysis of milk samples

The developed methodology was applied to the determination of Te species in cow and goat milk samples obtained from the Spanish market. These samples had different compositions, from full cream to skimmed milk, and contained also different additives, such as Omega 3, folic acid, Ca, minerals and vitamins, propolis or vegetable fat.

Results shown in Table 3 indicate the presence of free Te^{IV} concentrations from 0.6 to 6.5 $ng\,ml^{-1}$ and Te^{VI} levels from 0.4 to 4 $ng\,ml^{-1}$. In all samples, free Te^{IV} was the dominant specie in a nearly 3:2 ratio to the Te^{VI}.

Besides, total tellurium concentrations obtained by the proposed procedure for the samples analyzed are statistically comparable with those obtained after in batch dry-ashing microwave-assisted digestion or sonication following in all the cases by continuous HG-AFS determination.

The regression between data found by multicommutation and on-line reduction (y) and those obtained in batch after dry ashing (x_1) was $y = (1.02 \pm 0.04)x - (0.1 \pm 0.3)$ with $R^2 = 0.99$ and the regression between y and in batch measurement after pressurized microwave-assisted digestion (x_2) was $y = (1.00 \pm 0.02)x + (0.2 \pm 0.1)$ with $R^2 = 0.998$, thus indicating the good comparability of the fully mechanized developed methodology with continuous hydride generation after complete off-line digestion.

3.4. Analytical figures of merit

Table 4 shows the analytical features of HG-AFS Te determination in milk by using the batch and the multicommutation modes. Typical regression lines between peak height fluorescence measurements and Te concentrations were $I_F = (20 \pm 15) + (850 \pm 40)\ C_{Te}\ (ng\,ml^{-1})$ with a regression coefficient of $R = 0.999$ for batch mode and $I_F = (20 \pm 12) + (810 \pm 30)\ C_{Te}\ (ng\,ml^{-1})$ with $R = 0.998$ for the multicommutation approach. In the best conditions, detection limits of 0.021 and 0.023 $ng\,ml^{-1}$ were obtained for in batch and multicommutation, respectively. Thus, LODs of 0.52 and 0.57 $ng\,g^{-1}$ were found in terms of Te concentration in the original milk sample. So, it can be concluded that the main ana-

Table 3
HG-AFS determination of Te species in milk samples using slurries and both, multicommutation and batch modes

Milk sample	Total Te				Te^{IV}		Te^{VIc}	
	Multicommutation	Batch			Multicommutation	Batch	Multicommutation	Batch
		Sonication	Dry-ashing (Cava-Montesinos et al., 2003)[a]	MW (Cava-Montesinos et al., 2004)[b]				
Cow full cream	6.1 ± 0.2	6.2 ± 0.3	6.3 ± 0.3	6.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.2	2.2 ± 0.2	2.3 ± 0.3
Cow full cream with Ca	8.5 ± 0.4	8.3 ± 0.5	8.1 ± 0.7	8.2 ± 0.2	5.1 ± 0.5	4.9 ± 0.6	3.4 ± 0.4	3.4 ± 0.5
Cow partially skimmed	6.3 ± 0.8	6.4 ± 0.6	6.6 ± 0.3	6.2 ± 0.3	3.9 ± 0.5	3.9 ± 0.5	2.4 ± 0.5	2.5 ± 0.9
Cow skimmed with folic acid	9.0 ± 0.4	8.9 ± 0.4	8.7 ± 0.4	9.0 ± 0.1	5.7 ± 0.2	5.8 ± 0.4	3.3 ± 0.2	3.1 ± 0.3
Cow partially skimmed with vitamins and minerals	9.4 ± 0.5	9.1 ± 0.4	9.0 ± 0.3	9.3 ± 0.4	6.5 ± 0.4	6.3 ± 0.2	2.9 ± 0.5	2.8 ± 0.3
Cow skimmed with Omega 3	9.7 ± 0.6	9.5 ± 0.6	9.4 ± 0.7	9.2 ± 0.3	6.0 ± 0.6	5.8 ± 0.5	3.7 ± 0.5	3.7 ± 0.7
Cow skimmed	1.0 ± 0.2	1.2 ± 0.4	1.3 ± 0.7	0.8 ± 0.3	0.6 ± 0.3	0.8 ± 0.3	0.4 ± 0.3	0.4 ± 0.4
Cow vegetable base	2.8 ± 0.6	2.6 ± 0.7	2.4 ± 0.5	2.5 ± 0.3	1.9 ± 0.8	1.8 ± 0.6	0.9 ± 0.6	0.8 ± 0.5
Cow partially skimmed with propolis	8.9 ± 0.6	9.2 ± 0.7	9.3 ± 0.5	8.7 ± 0.1	5.3 ± 0.7	5.5 ± 0.7	3.6 ± 0.7	3.7 ± 0.7
Goat full cream	10.1 ± 0.2	10.4 ± 0.3	–	–	6.1 ± 0.1	6.3 ± 0.3	4.0 ± 0.2	4.1 ± 0.2

Te concentrations in milk are indicated in $ng\,ml^{-1}$, being also reported the standard deviation of three independent analysis of each sample.

[a] Data obtained after dry-ashing and batch HG-AFS (Cava-Montesinos et al., 2003).

[b] Data obtained after pressurized digestion by MW oven and batch HG-AFS (Cava-Montesinos et al., 2004).

[c] Te^{VI} concentrations were obtained from the difference between total tellurium and Te^{IV}.

Table 4
Analytical features of HG-AFS determination of Te by using batch and multicommutation modes

	Batch AFS	Multicommutation AFS
Calibration line[a]	$I_F = (20 \pm 15) + (850 \pm 40)\ C_{Te}$	$I_F = (20 \pm 12) + (810 \pm 30)\ C_{Te}$
Correlation coefficient (*R*)	0.999	0.998
RSD (%) [b]	11.2	10.5
RSD (%) [c]	5.4	6.3
LOD (ng ml^{-1})[d]	0.021	0.023
LOD (ng g^{-1})[e]	0.52	0.57
Sample consumption (ml)[f]	1700	318
Reagent consumption (ml)[f]	1300	324
Carrier blank consumption (ml)[f]	930	120
Ar consumption (ml min^{-1})	330	200
Waste (ml)[g]	1179	625
Throughput (h^{-1})	30[h]	82[h] 24[i]

[a] A minimum of four standards and a blank were used for each calibration line in the concentration range from 0 to 4 ng ml^{-1}. The average data were obtained from three calibration lines.

[b] RSD (%): The mean relative standard deviation was established from the average of the variation coefficient found for three replicate analysis of 10 commercially available samples.

[c] RSD (%): The relative standard deviation was established from five independent measurements of a solution containing 1.0 ng ml^{-1} of Te^{IV}.

[d] LOD: limit of detection concerning diluted samples.

[e] LOD: limit of detection regarding the original sample.

[f] Sample and reagent consumption corresponding to 100 analysis.

[g] Waste generated was established for 1 h working session.

[h] Throughput corresponding to HG-AFS detection system.

[i] Throughput corresponding to the microwave-assisted digestion of samples.

lytical parameters of the off-line sonication of milk samples followed by in batch reduction with KBr and continuous HG-AFS determination are comparable to those obtained by the same procedure but making the reduction of Te^{VI} to Te^{IV} on-line and using the multicommutation strategy for sample and reagents insertion in the experimental set-up.

Moreover, as Table 4 indicates, a sample volume of 3.18 ml and a reagent volume of 3.24 ml per analysis are enough to carry out the determination of tellurium by the developed procedure, being produced a waste of 625 ml for 82 fluorescence measurements, made in one hour, on using the multicommutation HG-AFS system. These parameters are clearly better than the 17.00 ml sample consumption and 13.00 ml reagent required for the batch measurements which also involves a waste generation of 1179 ml h^{-1} for only 30 measurements. So, it can be concluded that the methodology proposed in this study is a sustainable, fast and environmentally friendly alternative to previous traditional approaches.

Recovery experiments were made on several milk samples in order to evaluate the accuracy of the method and different concentrations of Te ^{IV} and Te^{VI} were added. As can be seen in Table 5, recovery values from 93 ± 7% to 104 ± 8% for Te^{IV} and from 94 ± 7% to 101 ± 2% for Te^{VI} were obtained, for ac-

Table 5
Recovery of Te^{IV} and Te^{VI} spiked concentrations added to different actual milk samples obtained by using both, the developed multicommutation approach an a traditional in batch reduction and continuous HG-AFS determination

Milk sample	Te^{IV} spiked	Te^{VI} spiked	Recovery % Te^{IV}		Recovery % Te^{VI}	
			Multicommutation	Batch	Multicommutation	Batch
Cow full cream	3	–	99 ± 6	101 ± 4	–	–
	–	3	–	–	101 ± 2	94 ± 7
	1.5	1.5	98 ± 4	95 ± 6	97 ± 5	96 ± 5
Cow partially skimmed	3	–	95 ± 8	96 ± 7	–	–
	–	3	–	–	98 ± 1	98 ± 2
	1.5	1.5	93 ± 7	94 ± 7	96 ± 4	96 ± 5
Cow skimmed with Omega 3	4	–	104 ± 8	102 ± 1	–	–
	–	4	–	–	97 ± 4	100 ± 4
	2	2	97 ± 2	99 ± 6	94 ± 7	95 ± 4
Cow partially skimmed with propolis	4	–	103 ± 4	100 ± 3	–	–
	–	4	–	–	98 ± 3	101 ± 5
	2	2	97 ± 2	97 ± 6	101 ± 2	99 ± 2

Tellurium recoveries (%) found in commercially available milk samples by using batch sonication and microwave-assisted pre-treatment. Measurements were carried out by batch and multicommutation modes. Standard deviation corresponding to three independent replicates. Te spikes in ng ml^{-1} were added before sample treatment.

tual samples spiked with a single one or both of the Te species considered, thus evidencing the absence of analyte losses or contamination during the sonication, the microwave-assisted reduction of samples and the hydride generation, evidencing also the lack of modification of the spiked species during the different analysis steps.

The regression between the two data populations obtained for Te^{IV} and Te^{VI} determination using in batch (x) and multicommutation (y) approaches (see Table 3 data) provided equations: $y = (1.01 \pm 0.03)x - (0.1 \pm 0.2)$ [Te^{IV}] and $y = (0.98 \pm 0.03)x + (0.05 \pm 0.09)$ [Te^{VI}] with regression coefficients of 0.996 and 0.996, respectively. Slope values of the aforementioned equation are comparable to 1 ($t_{\text{calculated}} = 0.484$ (Te^{IV}) and 0.690 (Te^{VI}), for a $t_{\text{theoretical}} = 1.734$, for 95% probability level and 18 freedom degrees). Ordinate values are around 0 ($t_{\text{calculated}} = 0.437$ (Te^{IV}) and 0.640 (Te^{VI}), for a $t_{\text{theoretical}} = 1.734$, for 95% probability level and 18 freedom degrees). So, it can be concluded that the on-line microwave-assisted reduction of Te^{VI} and multicommutation HG-AFS determination of free Te^{IV} and total Te does not modifie the accuracy of the traditional in batch and continuous HG-AFS determinations.

4. Conclusions

A non-chromatographic and sensitive method with good linearity, reproducibility, high sampling frequency and low waste generation has been proposed for speciation of inorganic tellurium in milk samples.

The on-line microwave-assisted reduction system reduces sample handling and makes possible the complete automation of the inorganic tellurium species determination. The use of a KBr solution in an acidic medium permits the quantitative reduction of Te^{VI} to Te^{IV} in less than 2 min, approximately, by using a microwave oven operating at 700 W.

Moreover, multicommutation minimizes the reagent consumption by a factor of 4 and the generation of toxic residues by a factor of 2 and improves the speed of analysis over three times as compared with the classical approaches. Thus, it is an economically sustainable and environmentally friendly alternative to previously reported methods.

Acknowledgements

Authors acknowledge the financial support from The Ministerio de Ciencia y Tecnología, Project No. AGL 2002-00729. Eva Ródenas acknowledges a "CINC SEGLES" grant from the Universitat de València of Spain.

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