Talanta 106 (2013) 66–72

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Multi-element analysis of milk by ICP-oa-TOF-MS after precipitation of calcium and proteins by oxalic and nitric acid

Lenka Husáková*, Iva Urbanová, Jitka Šrámková, Michaela Konečná, Jana Bohuslavová

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentska 573 HB/D, Pardubice 530 09, Czech Republic

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form
5 December 2012
Accepted 8 December 2012
Available online 14 December 2012

Keywords:
ICP-oa-TOF-MS
Trace elements
Milk
Interferences in inductively coupled plasma mass spectrometry
Precipitation
Oxalic acid

ABSTRACT

In this work a simple technique employing oxalic and nitric acid to cow's milk samples prior to analysis by inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry (ICP-oa-TOF-MS) was introduced. After the precipitation of calcium and proteins via oxalic and nitric acid, respectively, the resulting liquid phase was aspirated with a concentric glass nebulizer for ICP-TOF-MS determination of trace elements. Precipitation of proteins is essential for better separation of solid and liquid phase of modified samples. Separation of calcium as a precipitated non-soluble oxalate enables the elimination of spectral interferences originating from different calcium containing species like $^{40}Ca^{35}Cl^{+}$, $^{40}Ca^{37}Cl^{+}$, $^{43}Ca^{16}O^{+}$, $^{40}Ca^{18}O^{+}$, $^{44}Ca^{16}O^{+}$, $^{43}Ca^{16}O^{1}H^{+}$ onto the determination of As, Se, Co and Ni whose assay is more difficult when using conventional quadrupole instruments. High detection capability is further an advantage as the approach enables the analysis without dilution. The methodology may serve, in addition, for a fast and sensitive determination of some other elements. After that, direct, reliable and simultaneous determination of 16 elements (Li, Be, B, V, Cr, Mn, Ni, Co, Ga, As, Se, Mo, Sn, Sb, Cs, Tl) at trace and ultra-trace levels in milk can be performed under optimum instrumental conditions and by using Rh as an internal standard. Accuracy and precision was assessed by measuring NCS ZC73015 milk powder control standard, yielding results in agreement with certified values and RSD $<10\%$. The accuracy was also checked by comparison of the results of the proposed method with those found by a method based on a microwave-assisted digestion of real samples.

1. Introduction

The investigation of the element concentrations in milk is very important because of their nutritional and toxicological relevance in human health [1]. Due to the capability of multi-elemental analysis analytical procedures most commonly used involve inductively coupled plasma atomic emission spectrometry (ICP-AES) for monitoring of major elements [2,3] and ICP-MS for trace elemental and isotopic analysis [3,4].

Milk elemental ICP analysis by direct nebulisation of aqueous solutions is hampered by blockage of cones, deposition of organic matter in the injector tube of the torch, and spectral and non-spectral interferences [5]. The lack of accuracy and sensitivity was attributed to the fatty nature of milk [6], large drop size and the incomplete atomization in the plasma [7], and the chemical form of the analyte element as the variation in the degree of the atomization is expected during droplet residence in the plasma [2]. In order to overcome difficulties caused by the milk matrix for the ICP analysis different decomposition techniques [3] mainly with the use of microwave digestion were employed. However, in order to develop a simple, direct and quick procedure several authors [2,8,9] have continued in development of the methods and thus satisfactory results were obtained by aspirating 1+4 diluted milk whey samples into the ICP-MS [8], direct introduction of slurries obtained by employing a commercial mixture of water-soluble tertiary amines and EDTA to dilute milk samples [2] or by sonification of samples with mineral acids [9].

One of the main drawbacks in the analysis of milk by ICP-MS is the occurrence of spectral interferences due to polyatomic ions produced in plasma from argon and matrix constituents making the quantification of several trace elements difficult when systems with conventional low-resolution quadrupole mass discrimination are used [10–12]. The problem of spectral interferences can be overcome by using a reaction or collision cell ICP-MS instruments [5,10,13], but at the cost of a reduction of the multi-element capabilities. The use of sector field ICP-MS offers the capability to separate interfering molecules from the analyte ion [1,8,10,14] by a mass resolution up to 10,000. While losing sensitivity in the high resolution mode, the multi-element capability is being maintained. Nevertheless, these methods are less accessible to laboratories owing to the high cost of the instrumentation. Application of mathematical correction methods to

---

* Corresponding author. Tel.: +420 466 037 029; fax: +420 466 037 068.
E-mail address: Lenka.Husakova@upce.cz (L. Husáková).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.12.015

attenuate the interference to manageable level is less useful at ultra trace levels, digestion methods and conventional separation/preconcentration [15] may be relatively time consuming. However, completely automated methods involving an on-line combination of sorption preconcentration with the determination of elements by ICP-MS are characterized by high performance [16,17]. On the other hand, some difficulties of finding compromising conditions for analytes may occur for FI-ICP-MS methods, similarly as memory effects requiring a higher time for washing the column [17].

Modification of the sample preparation procedure by employing oxalic and nitric acid to cow's milk samples in order to separate calcium and proteins prior to ICP-TOF-MS analysis is introduced in this work. The main advantage of this approach is that it serves as a cheap, rapid and simple alternative for overcoming the spectral effects from different calcium based polyatomic species onto the determination of some elements (As, Se, Co and Ni) whose determination is difficult using conventional quadrupole instruments. Finally, the proposed method was proved to be accurate and precise for a multi-elemental determination of 16 trace and ultra-trace elements in milk.

2. Material and methods

2.1. Instrumentation

ICP-MS measurements were performed by the Optimass8000 ICP-oa-TOF-MS instrument (GBC Scientific Equipment Pty. Ltd., Australia) of which a detailed description is given in Ref. [18]. Details of the instrument and the operating conditions are summarized in Table 1. Using typical operating conditions sensitivity of 15,000 counts s^{-1} per μg L^{-1} (mass integrated peak) and resolution (full width at half maximum intensity) of 1800 was achieved for $^{139}La^+$. For $^{238}U^+$, the same parameters were 17,000 counts s^{-1} per μg L^{-1} and 2100, respectively.

Microwave digestion of samples was carried out in the Speedwave™ MWS-3^+ (Berghof, Germany) microwave system with the maximum total output of the microwave generator 1450 W. Centrifugation of the samples was performed using the NF 400 bench top centrifuge (NÜVE INC., Turkey).

The determination of Na, K, Ca and Mg was carried out with an Avanta P double beam atomic absorption spectrometer (GBC Scientific Equipment Pty. Ltd., Australia) in the flame emission and absorption mode, respectively. Sample solutions were introduced to the spectrometer through a standard GBC pneumatic nebulizer. The hollow cathode lamps obtained from Photron Pty. (Australia) were used throughout. The wavelength was set at 766.5 nm for K, 589.0 nm for Na, 422.7 nm for Ca and 285.2 nm for Mg with a lamp current of 3 mA. Spectral resolution of 0.2 and 0.5 nm was used for Na, K and Ca and Mg, respectively. The gas flame mixture was formed with 10.0 L min^{-1} air and 1.5 L min^{-1} acetylene (99.5%, from Linde Gas, Inc., Czech Republic).

2.2. Reagents and standards

All solutions were prepared using deionised water purified by means of the UltraClear (SG, Germany) pure water system to 0.05 μS cm^{-1}.

Sub-boiled nitric acid was prepared from nitric acid (65%, w/w) of Selectipur quality (Lach-Ner, Neratovice, Czech Republic). Calcium chloride used for interference studies was TraceSelect quality and obtained from Fluka (Fluka, Buchs, Switzerland). Rhodium internal standard solution was prepared from 1 g L^{-1} Rh solution obtained from SCP Science (Canada). Multi-element stock solution containing 10 mg L^{-1} of Li, B, Be, Al, Ti, V, Cr, Mn, Ni, Co, Cu, Ga, Ge, As, Se, Rb, Sr, Zr, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Te, Ba, Ta, W, Re, Pt, Hg, Tl, Pb, Bi, Th, U, Cs and Hf being routinely used in our laboratory was prepared from single element standards of 1 g L^{-1} obtained from Analytika Ltd. (Prague, Czech Republic) or SCP Science. The stock solutions of Na, K, Ca and Mg (1 g L^{-1}) were obtained from Analytika Ltd. (Prague, the Czech Republic).

Table 1
Instrumental operating conditions for ICP-TOF-MS

Instrument	GBC Optimass 8000
Sample uptake rate	1.0 mL min^{-1}
Sample introduction	Concentric nebulizer with cyclonic spray chamber (70 mL, 10 °C)
ICP Source	
Rf power (27.12 MHz)	1250 W
Plasma gas flow rate	11 L min^{-1}
Auxiliary gas flow rate	0.600 L min^{-1}
Nebuliser gas flow rate	0.980 L min^{-1}
Mass spectrometer	
Ion optics	
Sampler cone	Nickel
Skimmer cone	Nickel-plated copper
Extraction	−1300 V
Z1	−190 V
Y mean	−495 V
Y deflection	−1 V
Z lens mean	−1050 V
Z lens deflection	−35 V
Lens body	−140 V
Pulse shaping	
Fill	−39 V
Fill bias	−0.1 V
Fill grid	−2.0 V
Pushout plate	577 V
Pushout grid	−473 V
Blanker	200 V
Spectral frequency	33 kHz
Reflectron	600 V
Detection	
Multiplier gain	2630 V
Ion threshold	8.4 mV
Integration window	Auto
Measurement mode	Pulse counting/analog
SMARTGATE ion blanking system	11.5–48.5 AMU ($^{12}C^+$, $^{14}N^+$, $^{16}O^+$, $^{17}OH^+$, $^{18}H_2O^+$, $^{28}N_2^+$, $^{30}NO^+$, $^{32}O_2^+$, $^{32}S^+$, $^{40}Ar^+$, $^{41}ArH^+$, $^{80}Ar^{2+}$) 55.5–57.5 AMU ($^{56}ArO^+$, $^{56}Fe^+$) 79.4–81.7 AMU ($^{40}Ar_2^+$) 84.5–87.5 AMU ($^{85,87}Rb^+$)

2.3. Quality assurance and quality control

The commercially supplied certified reference material (CRM) NCS ZC73015 milk powder obtained from National Research Centre for Certified Reference Materials (China) was used for the assessment of the accuracy and precision of the concentration of trace elements determined in milk samples.

2.4. Samples

Commercially available samples of milk were selected to represent whole (3.5% w/v fat), skimmed (0.5% w/v fat), and low-fat (1.5% w/v fat) liquid cow's milk samples.

2.5. Sample preparation

2.5.1. Precipitation

About 500 mg of the milk CRM was weighed into a beaker and reconstituted completely with 5 mL of deionized water at elevated temperature (≈ 50 °C) using a pre-heated water bath. Since only small amount of the sample was used, the warm up of the samples took only about 1 min. Then, the sample was transferred into a centrifuge tube, in which Ca as oxalate was precipitated by adding 350 μL of 50 g L^{-1} oxalic acid solution to the sample immediately followed by addition of 100 μL of 65% HNO_3 in order to precipitate the proteins, thus ensuring an easy filtration of the sample which followed after the centrifugation. The sample was manually shaken for 30 s and then the contents were centrifuged for 10 min at 3500 rpm. In order to protect the possible blockage of the interface the liquid phase was transferred to a polyethylene syringe without disturbing the precipitate and filtered through a 0.45 μm syringe filter (Whatman Autovial) to a 2-mL polyethylene tube.

When the liquid milk samples were prepared, 5 mL of milk was pipetted in general directly into a centrifuge tube, the samples were heated up to $\approx 50\,^{\circ}C$ using a pre-heated water bath and the precipitation was performed using the same way as described above.

2.5.2. *Microwave assisted digestion*

A powdered milk sample aliquot of 0.5 g or 5 mL of liquid milk sample was accurately weighed into a 100-mL pressure resistant PTFE vessel. Then 5 mL of HNO_3 (65%, m/v) and 2 mL of H_2O_2 (30%, m/v) were added and the vessels were allowed to stand open until the initial reaction subsided. The samples were digested following a five-step program: (i) 5 min at 160 °C and 80% power (ramp 3 min), (ii) 10 min at 220 °C and 90% power (ramp 5 min), (iii)–(v) 5 min at 100 °C and 10% power (ramp 1 min). The resulting colourless solutions were diluted to 25 mL with deionized water. Each sample was decomposed in three replicates. Blanks, consisting of deionised water and reagents were subjected to a similar sample preparation procedure.

2.6. *Sample analysis*

2.6.1. *Analysis by ICP-MS*

Samples and standards treated by different ways, as indicated before, were introduced into the ICP-MS. Blank, standard and milk sample solutions were nebulized followed by introduction of deionised water for at least 1 min to rinse the sampling system in order to avoid the contamination of other solutions or samples. Quantification of trace element concentrations was performed establishing calibration curves by linear regression. Aqueous multi-element standard solutions prepared daily from multi-element standard of 500 μg L^{-1} were used for calibration as the differences of the calibration slopes of milk and acid based standards were less than 10%. The concentrations of the elements were as follows: blank, 1, 2, 5, 10 and 20 μg L^{-1} of Li, B, Be, Al, Ti, V, Cr, Mn, Ni, Co, Cu, Ga, Ge, As, Se, Rb, Sr, Zr, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Te, Ba, Ta, W, Re, Pt, Hg, Tl, Pb, Bi, Th, U, Cs, Hf. To compensate the possible instrumental drift and matrix effects, all samples and solutions contained 0.2 μg L^{-1} of Rh. Regression coefficients greater than 0.999 were obtained regularly. Peak area mode and 10 s data acquisition time and ten replicates were used for the measurement.

2.6.2. *Analysis by flame atomic absorption/emission spectrometry (FAAS/FAES)*

A single calibration graph was constructed for each element, from which all calculations were made in one analytical run. Five aqueous standards were used to obtain the calibration curves. The concentration ranges of calibration solutions were 0–20 mg L^{-1} and 0–0.4 mg L^{-1} for Na, K, Ca, and Mg, respectively. Five replicates at three-second integration time were used. All calibration plots were linear in the investigated concentration ranges with the correlation coefficients better than 0.999.

3. Results and discussion

3.1. *Effect of oxalic acid on the removal of spectral interference*

Although it was previously shown that precipitation of chloride matrix can effectively reduce chloride based interferences [19] and the precipitation of Ca as an oxalate may overcome interferences from CaO^+ species on the determination of nickel by ICP-MS [20], this approach is not commonly used in ICP-MS. Currently, in spectrochemical practice the oxalic precipitation is well established in a large scale separation/preconcentration procedures during the determination of rare earths [21] and also a method for the measurement of calcium isotope ratios ($^{42}Ca/^{43}Ca$ and $^{44}Ca/^{43}Ca$) in urine using DRC-ICP-MS after oxalate precipitation was performed [22]. The use of oxalic acid in ICP-MS determination has also been proved to increase the sensitivity of analytes, especially for the elements with ionization potential between 9 and 11 eV, to improve the detection limits and can be used to compensate the signal depression caused by inorganic matrix [23]. However, according to the best of our knowledge, with the exception of the use of oxalate precipitation to avoid CaO^+ spectral polyatomic interferences onto the determination of Ni in serum analysis [20], no studies were performed relating the use of oxalic acid to overcome the spectral effects from different calcium containing species for different analytes. In addition, normal calcium range in serum varies by individual labs 80–110 mg L^{-1} [24,25] while calcium levels in common cow's milk samples are typically up to 1300 mg L^{-1} [26–28]. Relating the analysis of cow's milk, the interference is thus expected to be more serious in contrast to serum samples.

In the analysis of food matrices, polyatomic species involving mainly Na, K, Ca, Mg, S, Cl, P and C were found more or less important relating the determination of elements below 100 AMU [10,29] unless at least a medium resolution power ($m/\Delta m = 3000$) is applied [10]. Quantitative data of apparent elemental concentration for a different matrix element within the range of 25–1000 mg L^{-1} encountered on the Optimass 8000 were recently reported [30]. Interferences from different calcium containing species like $^{40}Ca^{18}O^+$, $^{44}Ca^{16}O^+$, $^{43}Ca^{16}O^1H$ or $^{43}Ca^{16}O^+$ were observed for $^{58}Ni^+$, $^{60}Ni^+$ or $^{59}Co^+$. The presence of $^{40}Ca^{35}Cl^+$ and $^{40}Ca^{37}Cl^+$ was found to be more significant for determination of $^{75}As^+$ and $^{77}Se^+$, respectively rather than $^{40}Ar^{35}Cl^+$ and $^{40}Ar^{37}Cl^+$, which are the most widely debated interferences in the literature [11,12] relating the determination of As and Se in different samples.

Influence of Ca^{2+} (chloride) amount within the range of 0–565 mg L^{-1} on the relative concentration signal of 5 μg L^{-1} of $^{60}Ni^+$, $^{75}As^+$ and $^{77}Se^+$ as the most interfered isotopes from the multi-elemental standard in the absence and in the presence of oxalic acid is shown in Fig. 1. It can be seen from the data presented in Fig. 1, that the presence of oxalic acid effectively suppresses the interference from different Ca containing species both onto $^{60}Ni^+$, as described previously [20], but also for other interfered elements like $^{75}As^+$ and $^{77}Se^+$. Similarly, the suppression of the above mentioned interference was observed for $^{58}Ni^+$, $^{61}Ni^+$, $^{62}Ni^+$ and $^{59}Co^+$.

3.2. *Accuracy and precision*

A certified reference milk powder NCS ZC73015 was analysed to test the accuracy and precision of the proposed method after precipitation of the sample with oxalic acid and nitric acid. The results for mineralized samples, i.e. using the most established way for the milk samples preparation, are also included for comparison purposes especially for the cases when only indicative values for several elements were given by the producer of the

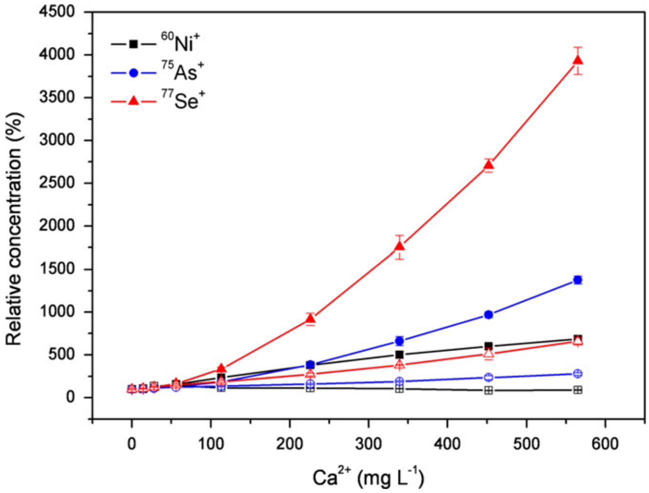

Fig. 1. Influence of Ca^{2+} (chloride) amount on the relative concentration signal of 5 μg L^{-1} of $^{60}Ni^+$, $^{75}As^+$ and $^{77}Se^+$ from multi-elemental standard in the absence (closed symbols) and in the presence of oxalic acid (open symbols). Relative concentration=concentration evaluated for 5 μg L^{-1} multi-elemental standard in the presence of oxalic acid and Ca^{2+} within the investigated range/concentration evaluated for 5 μg L^{-1} standard at conditions without the interfering matrix element. Bars indicate the range for each determination (n=10).

reference material. It can be seen from the data presented in Table 2 that good agreement was achieved between the certified values and those determined by ICP-TOF-MS for the 8 elements reported. Additionally, for 5 elements good agreement was achieved between the results obtained by the proposed method and indicative value and/or those found after microwave digestion method (see Table 2). As many ions form non-soluble oxalates in water [31], the precipitation will influence not only the determination of calcium, whose content in the original CRM NCS ZC73015 milk powder and different cow's milk samples and those samples after the precipitation is shown in Tables 2 and 3, respectively, but relating the elements being present in the multi-element standard (See Section 2.2) also the determination of Sr, Cu, Bi, Pb, Ag, Th or Hg. Co-precipitation and/or inclusion of the analytes in the precipitated matrix may influence the determination of some other elements which do not form non-soluble oxalates like Cd, Ba, etc. for which the under estimated values were observed (data not shown here). On the other hand, the determination of usually troublesome elements like $^{75}As^+$, $^{77}Se^+$, $^{59}Co^+$, $^{51}V^+$, $^{58,60}Ni^+$, $^{53}Cr^+$ requiring a collision/reaction cells in the lack of an adequate resolution method, using the proposed method can be performed more easily without interference. Regarding the determination of As, Se, Co, Ni the elimination of interferences from those of $^{40}Ca^{35}Cl^+$, $^{40}Ca^{37}Cl^+$, $^{40}Ca^{18}O^+$, $^{43}Ca^{16}O^+$, $^{44}Ca^{16}O^+$, etc. species is expected, the non-significant effect of the chloride based interferences onto $^{53}Cr^+$, or $^{51}V^+$ is questionable. On the other hand, the interference from carbon and sulphur containing species did not allow the quantification of $^{52}Cr^+$ and $^{49}Ti^+$, etc. Similarly, as the precipitation by oxalic acid does not influence the determination of Na, K or Mg as can be seen from the data presented in Tables 2 and 3, respectively, for the analysis of CRM NCS ZC73015 milk powder and different cow's milk samples, the interference from polyatomic species containing these elements occurs e.g. during the determination of nickel at the mass $^{61}Ni^+$ or $^{62}Ni^+$.

Other elements being certified in the NCS ZC73015 milk powder and present in the calibration standards like Cs, Sb, Tl whose determination is not prone to spectral effects during the analysis of milk can be in addition quantified accurately by the proposed method.

Table 2
Comparison of determined and certified or indicative concentrations in NCS ZC73015 milk control standard evaluated after microwave digestion and precipitation with oxalic acid and intra- and inter-day variation coefficients (VCs).

	Certified (μg g^{-1})	Found[a] (μg g^{-1})		VC[f] (%)	
		Microwave digestion	Precipitation	Intra-day	Inter-day
ICP-TOF-MS					
^{7}Li	0.04[b]	0.041 ± 0.005	0.041 ± 0.005	6.1	3.1
^{11}B	1.56 ± 0.22	1.04 ± 0.31	1.62 ± 0.23	7.1	10.7
^{51}V	0.06[b]	0.059 ± 0.004	0.066 ± 0.004	3.0	12.1
^{53}Cr	0.39 ± 0.04	0.40 ± 0.09[e]	0.35 ± 0.015	2.1	10.9
^{55}Mn	0.51 ± 0.17	0.30 ± 0.06	0.31 ± 0.03	4.8	7.8
^{58}Ni	0.18[b]	0.209 ± 0.033	0.177 ± 0.01	2.8	6.3
^{59}Co	0.030 ± 0.007	0.030 ± 0.004	0.026 ± 0.003	5.8	10.7
^{75}As	0.031 ± 0.007	0.036 ± 0.004	0.038 ± 0.004	5.3	13.8
^{77}Se	0.11 ± 0.03	0.126 ± 0.009	0.14 ± 0.007	2.5	8.0
^{95}Mo	0.28 ± 0.03	0.26 ± 0.01	0.24 ± 0.015	3.1	8.0
^{121}Sb	0.006[b]	0.0067 ± 0.002	0.0073 ± 0.0008	5.2	8.0
^{133}Cs	0.034 ± 0.005	0.035 ± 0.002	0.030 ± 0.004	6.7	6.6
^{205}Tl	0.9[b,c]	0.64 ± 0.21[c]	0.97 ± 0.07[c]	3.6	13.6
FAES/FAAS					
Na	4.7 ± 0.3[d]	4.3 ± 0.4[d]	4.6 ± 0.2[d]	2.2	7.5
K	12.5 ± 0.5[d]	12.45 ± 0.02[d]	12.3 ± 0.9[d]	3.7	8.0
Ca	9.4 ± 0.3[d]	9.46 ± 0.02[d]	0.80 ± 0.03[d]	1.9	10.8
Mg	0.96 ± 0.07[d]	0.92 ± 0.04[d]	0.90 ± 0.06[d]	3.3	6.9

[a] Mean ± 2 S.D. (n=3).
[b] Indicative concentration.
[c] ng g^{-1}.
[d] mg g^{-1}.
[e] Isotope $^{52}Cr^+$ was used for quantification.
[f] Values evaluated for the precipitated samples.

The precision of the method was evaluated in terms of intra- and inter-day comparison. Intra-day precision was determined by analysis of milk control material three times during the same day. Inter-day precision was determined by analysis of the same standards on three different days over a period of one month. Within each series, every solution was analysed in ten replicates. Relative standard deviation (RSD) was calculated for both series of analyses. The RSD values of intra- and inter-day studies typically found to be below 14% show that the precision of the method was satisfactory (see Table 2).

3.3. *Analysis of real samples*

Three different cow's milk samples (skimmed milk, low-fat milk, and whole milk) were analysed by ICP-TOF-MS after the precipitation with oxalic and nitric acid and after the conventional microwave digestion method [29]. The results obtained for the analysis are shown in Table 3.

Linear regression was applied to method comparison data as it provides useful information about proportional, constant, and random error via, respectively, the slope, intercept, and standard deviation of the residuals ($S_{y/x}$) [32]. A regression line between comparative values (x) obtained by microwave digestion and results (y) was $y=(1.000 \pm 0.023)\ x - (0.365 \pm 0.753)$ with $R^2=0.998$ for a skimmed milk sample, $y=(1.036 \pm 0.037)\ x - (1.144 \pm 1.188)$ with $R^2=0.996$ for a low-fat milk sample, and $y=(1.001 \pm 0.053)\ x \pm (0.899 \pm 1.886)$ with $R^2=0.991$ for a whole milk sample, respectively. Additionally, paired Student's t-tests were applied for comparison of the results obtained by both methods at the significance level of 0.05. The results obtained using both procedures for the determination of 16 elements in total were not significantly different for a 95%

Table 3
Concentrations [a] (μg L^{-1}) of trace elements in raw cow's milk samples obtained using different sample treatments: precipitation and microwave digestion (MWD)

	Skimmed milk (0.5% w/v fat)		Low-fat milk (1.5% w/v fat)		Whole milk (3.5% w/v fat)	
	MWD	Precipitation	MWD	Precipitation	MWD	Precipitation
ICP-TOF-MS						
^{7}Li	2.9 ± 0.2	3.0 ± 0.2	2.2 ± 0.3	2.6 ± 0.2	3.5 ± 0.7	3.6 ± 0.3
^{9}Be	0.26 ± 0.04	0.24 ± 0.02	0.34 ± 0.10	0.24 ± 0.07	0.42 ± 0.13	0.26 ± 0.08
^{11}B	106 ± 9	105 ± 5	113 ± 13	118 ± 9	122 ± 4	122 ± 7
^{51}V	7.9 ± 0.3	7.5 ± 0.6	11.2 ± 0.6	10.2 ± 0.9	8.3 ± 0.2	8.9 ± 0.6
^{53}Cr	51 ± 3[b]	52 ± 8	29 ± 2[b]	27 ± 2	44 ± 3[b]	48 ± 2
^{55}Mn	15.9 ± 0.6	13.2 ± 0.7	15 ± 1	16 ± 2	14.2 ± 0.7	13.5 ± 0.6
^{58}Ni	16.3 ± 0.7	14 ± 3	19 ± 1	15 ± 3	22 ± 7	12 ± 4
^{59}Co	3.7 ± 0.3	3.2 ± 0.3	4.2 ± 1.2	3.4 ± 1.0	3.6 ± 1.1	2.7 ± 0.8
^{69}Ga	2.9 ± 0.2	2.8 ± 0.9	2.4 ± 0.1	2.3 ± 0.2	3.2 ± 0.3	3.5 ± 0.4
^{75}As	13 ± 2	12.1 ± 0.6	17 ± 2	15 ± 1	15.6 ± 1.3	13.7 ± 0.8
^{77}Se	35 ± 2	34 ± 2	33 ± 4	33 ± 2	35 ± 1	33 ± 2
^{95}Mo	42 ± 2	44 ± 18	29 ± 2	25 ± 2	33 ± 1	30 ± 5
^{118}Sn	0.76 ± 0.08	0.69 ± 0.28	0.73 ± 0.11	0.56 ± 0.07	0.70 ± 0.06	0.59 ± 0.07
^{121}Sb	0.12 ± 0.03	0.17 ± 0.02	0.14 ± 0.02	0.16 ± 0.02	0.19 ± 0.06	0.22 ± 0.03
^{133}Cs	2.4 ± 0.7	2.5 ± 0.2	3.4 ± 0.2	3.0 ± 0.2	2.5 ± 0.1	2.3 ± 0.1
^{205}Tl	0.18 ± 0.01	0.19 ± 0.02	0.135 ± 0.010	0.11 ± 0.03	0.14 ± 0.02	0.11 ± 0.01
FAES/FAAS						
Na	441 ± 10[c]	446 ± 7[c]	426 ± 12[c]	420 ± 10[c]	433 ± 20[c]	442 ± 10[c]
K	1639 ± 20[c]	1655 ± 95[c]	1570 ± 25[c]	1600 ± 32[c]	1609 ± 35[c]	1587 ± 30[c]
Ca	1313 ± 26[c]	148 ± 4[c]	1028 ± 12[c]	84 ± 4[c]	1023 ± 4[c]	99 ± 10[c]
Mg	105 ± 2[c]	111 ± 7[c]	103 ± 2[c]	106 ± 6[c]	104 ± 2[c]	107 ± 4[c]

[a] Mean ± 2S.D. (n=3).
[b] Isotope ^{52}Cr^{+} was used for quantification.
[c] mg L^{-1}.

confidence level (p=0.110, p=0.169, and p=0.120 for samples of skimmed milk, low-fat milk, and whole milk, respectively) and thus both methods are comparable.

3.4. *Limits of detection and sensitivity*

Detection limits (LODs) for each isotope, based on three times the standard deviation of the blank response at the given m/z position divided by the slope of the calibration graph are given in Table 4 for original milk samples and both sample preparation procedures, i.e., precipitation and microwave digestion. The integration time was set to 10 s and standards and blanks were determined with 10 replicates. The blank consisted of ultrapure water, nitric acid and hydrogen peroxide and Rh as an internal standard at their operational concentrations for the former case (MWD) and of ultrapure water, oxalic acid and Rh for the latter case (precipitation). Significantly higher detection power is evident from the data presented in Table 4 when using precipitation in contrast to MWD method. LODs obtained by the proposed method were also mostly better or comparable as those previously reported by several authors in the absence of polyatomic interferences, using different approaches in various milk sample analysis and using Q-ICP-MS [9], DF-ICP-MS [8,10,14] or DRC-ICP-MS [5,13] instrumentation (see Table 4). On the other hand, the LODs values were poorer for some elements like Cr, Mo, Ni due to possible contamination originating from the reagents or introduction system materials, from previously introduced solutions (memory effect) or the use of low abundance isotope, etc. It is also interesting to compare the proposed procedure with FI-ICP-MS methods based on the on-line preconcentration of the analytes using a mini-column filled with C18 immobilized on silica to retain the complexes formed with the ammonium salt of *O*, *O*-diethyl dithiophosphoric acid and elution with methanol, being previously reported [17,33] for the determination of different analytes in various biological samples and offering a high sensitivity, high performance and detection power, good precision, elimination of polyatomic interferences or complete automation of the analysis cycle. Relating the LODs obtained the precipitation method may be comparable or somewhat better depending on the concrete analyte due to the analysis without dilution. Although for FI-ICP-MS high preconcentration is achieved depending on the analyte [17,33], dilutions factors due to the digestion of the sample may significantly influence the LODs values.

3.5. *Sample throughput*

Simultaneous analysis of all elements took for each sample (including ten replicate measurements and rinsing) approximately three minutes under the conditions described in Table 1, i.e., ca 20 samples could be measured within an hour. Microwave digestion of powdered material took about one hour for eight samples including weighing, transfer, and dilution of the digestates. Preparation of solid samples using precipitation method took approximately 40 min for the same number of samples including weighing, reconstitution, centrifugation, and filtration. The preparation of liquid samples was shorter because time consuming weighing (and reconstitution) was replaced by pipetting. For liquid samples, the whole microwave digestion procedure took about 55 min, the precipitation method approximately 35 min. As can be seen, the precipitation method offers the possibility to prepare the same number of samples in much shorter time as is needed for the microwave digestion. However, the time needed for the sample preparation may vary between laboratories depending on the instrumentation available both for the microwave digestion and for the precipitation method.

4. Conclusions

The proposed milk sample preparation with oxalic and nitric acid used for precipitation of calcium and proteins is a simple technique for accurate determination of 16 elements in milk by ICP-MS method. The method was validated for analysis of a

Table 4
Limits of detection for analysis of different elements in original milk samples with the use of Rh as internal standard after microwave digestion (MWD) and precipitation.

	Powdered samples[a]		Liquid samples		Reported LOD [Ref.][b] (Method) [d] (ng L^{-1})
	MWD (ng g^{-1})	Precipitation (ng g^{-1})	MWD (ng L^{-1})	Precipitation (ng L^{-1})	
^{7}Li	0.68	0.08	68	7.7	50 [9] (Q-ICP-MS)
^{9}Be	1.7	0.28	170	28	
^{11}B	72	7.9	7180	790	
^{51}V	1.08	0.31	108	31	16 [5] (DRC-ICP-MS) 70 [9] (Q-ICP-MS)
^{53}Cr	10.6	1.7	1060	170	99 [5] (DRC-ICP-MS) 100 [8] (DF-ICP-MS) 90 [9] (Q-ICP-MS) 210 [10] (DF-ICP-MS) 25 [13] (DRC-ICP-MS)
^{55}Mn	0.58	0.07	58	7.1	103 [5] (DRC-ICP-MS) 50 [8] (DF-ICP-MS) 30 [9] (Q-ICP-MS) 900 [10] (DF-ICP-MS)
^{58}Ni	7.5	0.34	750	34	1500 [8] (DF-ICP-MS) 6 [9] (Q-ICP-MS) 7500 [10] (DF-ICP-MS)
^{59}Co	0.15	0.03	15	3.1	6 [9] (Q-ICP-MS)
^{69}Ga	0.43	0.05	43	5.0	
^{75}As	2.3	0.39	230	39	80 [9] (Q-ICP-MS) 120 [13] (DRC-ICP-MS) 3.04[c] [14] (DF-ICP-MS)
^{77}Se	44	7.0	4376	700	1000 [8] (DF-ICP-MS) 30 [9] (Q-ICP-MS) 7800 [10] (DF-ICP-MS)
^{95}Mo	5.4	0.97	540	97	30 [9] (Q-ICP-MS)
^{118}Sn	0.74	0.10	74	10.3	10 [9] (Q-ICP-MS)
^{121}Sb	0.47	0.08	47	7.8	80 [9] (Q-ICP-MS)
^{133}Cs	0.08	0.01	8.3	1.2	8 [9] (Q-ICP-MS)
^{205}Tl	0.08	0.02	7.7	1.7	

[a] Sample mass of 0.5 g used for calculations.

[b] Values are reported for a given element, however, in general any isotope, if available, could be used. The values for liquid and powdered samples are expressed in ng L^{-1} and ng g^{-1}, respectively.

[c] ng g^{-1}.

[d] DF-ICP-MS: Double focusing sector field inductively coupled plasma mass spectrometry. DRC-ICP-MS: Dynamic reaction cell inductively coupled plasma mass spectrometry. Q-ICP-MS: Quadrupole ICP-MS.

normal cow's milk samples with calcium content up to 1300 mg L^{-1}. One advantage is that using this approach the interference of the matrix is decreased significantly and accurate analysis with the use of simple calibration method can be performed even for elements like As, Se, Co or Ni which are more troublesome if conventional Q-ICP-MS instruments are used. Moreover no use of a collision/reaction cell to attenuate the interferences to a manageable level, or the use of very costly high resolution instrumentation, previously reported for its quantification, is needed. Another benefit of this procedure is its simplicity and speed in contrast to conventional digestion methods during which, in addition, elements can be lost when the sample is heated. All the elements are determined simultaneously without the need of a specific setting for individual analytes which may be another benefit over Q-ICP-MS or FI-ICP-MS methods. Additionally, high detection capability is another advantage as the approach enables the analysis without dilution. However, the detection limits are limited by the quality of the blank solution, mainly by oxalic acid. On the other hand, some elements like Cd, Pb, Hg, Sr, Cu, Bi, Ag, etc. cannot be measured properly in milk samples with the proposed methodology due to the problems with co-precipitation and/or inclusion in the precipitated matrix. This problem is not observed when the samples are microwave digested. This procedure thus is not aimed to replace the most powerful microwave digestion, but can help to easily solve some of the above mentioned problems. This represents a meaningful improvement to earlier limited milk analysis procedures described.

Acknowledgements

Financial support from the Ministry of Education, Youth and Sports of the Czech Republic (project no. 0021627502) is gratefully acknowledged.

References

[1] M. Krachler, T. Prohaska, G. Koellensperger, E. Rossipal, G. Stingeder, Biol. Trace Elem. Res. 76 (2000) 97–112.

[2] J.A. Nobrega, Y. Gelinas, A. Krushevska, R.M. Barnes, J. Anal. At. Spectrom. 12 (1997) 1243–1246.

[3] M.D.A. Korn, E.S.D. Morte, D.C.M.B. dos Santos, J.T. Castro, J.T.P. Barbosa, A.P. Teixeira, A.P. Fernandes, B. Welz, W.P.C. dos Santos, E.B.G.N. dos Santos, M. Korn, Appl. Spectrosc. Rev. 43 (2008) 67–92.

[4] S.F. Durrant, Trends Anal. Chem. 11 (1992) 68–73.

[5] A. Ataro, R.I. McCrindle, B.M. Botha, C.M.E. McCrindle, P.P. Ndibewu, Food Chem. 111 (2008) 243–248.

[6] S.E. Emmett, J. Anal. At. Spectrom. 3 (1988) 1145–1146.

[7] E. Coni, A. Stacchini, S. Caroli, P. Falconieri, J. Anal. At. Spectrom. 5 (1990) 581–586.

[8] F.A. Rivero Martino, M.L. Fernandez Sanchez, A. Sanz Medel, J. Anal. At. Spectrom. 15 (2000) 163–168.

[9] P. Cava-Montesinos, M.L. Cervera, A. Pastor, M. de la Guardia, Anal. Chim. Acta 531 (2005) 111–123.

[10] F.A. Rivero Martino, M.L. Fernandez Sanchez, A. Sanz-Medel, Anal. Chim. Acta 442 (2001) 191–200.

[11] E.H. Evans, J.J. Giglio, J. Anal. At. Spectrom. 8 (1993) 1–18.

[12] N.M. Reed, R.O. Cairns, R.C. Hutton, Y. Takaku, J. Anal. At. Spectrom. 9 (1994) 881–896.

[13] S. D'Ilio, F. Petrucci, M. D'Amato, M. Di Gregorio, O. Senofonte, N. Violante, Anal. Chim. Acta 624 (2008) 59–67.

[14] C. Frazzoli, B. Bocca, Microchim. Acta 162 (2008) 43–50.

[15] S.F. Durrant, A. Krushevska, D. Amarasiriwardena, M.D. Argentine, S. Romon-Guesnier, R.M. Barnes, J. Anal. At. Spectrom. 9 (1994) 199–204.
[16] M.A. Bolshov, V.K. Karandashev, G.I. Tsisin, Y.A. Zolotov, J. Anal. Chem. 66 (2011) 548–564.
[17] V.L. Dressler, D. Pozebon, A.J. Curtius, Anal. Chim. Acta 438 (2001) 235–244.
[18] R.E. Sturgeon, J.W.H. Lam, A. Saint, J. Anal. At. Spectrom. 15 (2000) 607–616.
[19] T.D.B. Lyon, G.S. Fell, R.C. Hutton, A.N. Eaton, J. Anal. At. Spectrom. 3 (1988) 265–271.
[20] S.X. Xu, L. Stuhne-Sekalec, D.M. Templeton, J. Anal. At. Spectrom. 8 (1993) 445–448.
[21] K. Iwasaki, H. Haraguchi, Anal. Chim. Acta 208 (1988) 163–172.
[22] S. Sturup, L. Bendahl, B. Gammelgaard, J. Anal. At. Spectrom. 21 (2006) 297–304.
[23] X.D. Nie, X.M. He, L.B. Li, H.L. Xie, Spectrosc. Spect. Anal. 27 (2007) 1420–1423.
[24] A. Pizent, J. Jurasovic, S. Telisman, J. Trace Elem. Med. Biol. 17 (2003) 199–205.
[25] M. Peacock, Clin. J. Am. Soc. Nephrol. 5 (2010) S23–S30.
[26] M.A. de la Fuente, M. Juarez, Analyst 120 (1995) 107–111.
[27] G.V. Pashkova, Food Anal. Methods 2 (2009) 303–310.
[28] G.C. Brandao, G.D. Matos, S.L.C. Ferreira, Microchem. J. 98 (2011) 231–233.
[29] L. Husakova, I. Urbanova, J. Sramkova, T. Cernohorsky, A. Krejcova, M. Bednarikova, E. Frydova, I. Nedelkova, L. Pilarova, Food Chem. 129 (2011) 1287–1296.
[30] L. Husakova, I. Urbanova, L. Audrlicka-Vavrusova, J. Sramkova, T. Cernohorsky, M. Bednarikova, L. Pilarova, Microchim. Acta 173 (2011) 173–181.
[31] D.R. Lide, H.P.R. Frederikse (Eds.), CRC Handbook of Chemistry and Physics, 75th ed.,CRC, Boca Raton, FL, 1994.
[32] D. Stockl, K. Dewitte, L.M. Thienpont, Clin. Chem. 44 (1998) 2340–2346.
[33] V.L. Dressler, D. Pozebon, A.J. Curtius, Spectrochim. Acta Part B 53 (1998) 1527–1539.