Single-step solubilization of milk samples with N,N-dimethylformamide for inductively coupled plasma-mass spectrometry analysis and classification based on their elemental composition

Silvana M. Azcarate [a], Marianela Savio [b,1], Patricia Smichowski [c,d], Luis D. Martinez [b], José M. Camiña [a], Raúl A. Gil [b,*]

[a] Instituto de las Ciencias de la Tierra y Ambientales de La Pampa (INCITAP), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Av. Uruguay 151, L6300XAI Santa Rosa, La Pampa, Argentina
[b] Instituto de Química de San Luis (CCT-San Luis) – Área de Química Analítica, Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, D5700BWQ San Luis, Argentina
[c] Comisión Nacional de Energía Atómica, Gerencia Química, Av. Gral Paz 1499, B1650KNA San Martín, Buenos Aires, Argentina
[d] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, C1033AAJ Ciudad de Buenos Aires, Argentina

ARTICLE INFO

ABSTRACT

A single-step procedure for trace elements analysis of milk samples is presented. Solubilization with small amounts of dymethylformamide (DMF) was assayed prior to inductively coupled plasma mass spectrometry (ICPMS) detection with a high efficiency sample introduction system. All main instrumental conditions were optimized in order to readily introduce the samples without matrix elimination. In order to assess and mitigate matrix effects in the determination of As, Cd, Co, Cu, Eu, Ga, Gd, Ge, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sm, S, Sr, Ta, Tb, V, Zn, and Zr, matrix matching calibration with ^{103}Rh as internal standard (IS) was performed. The obtained limits of detection were between 0.68 (Tb) and 30 (Zn) $\mu g \, L^{-1}$. For accuracy verification, certified Skim milk powder reference material (BCR 063R) was employed. The developed method was applied to trace elements analysis of commercially available milks. Principal components analysis was used to correlate the content of trace metals with the kind of milk, obtaining a classification according to adults, baby or baby fortified milks. The outcomes highlight a simple and fast approach that could be trustworthy for routine analysis, quality control and traceability of milks.

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1. Introduction

Milk is a vital food in the human diet due to its high nutritional value and health benefits. Habitual consumption is recommended during all stages of development and life, especially for infants and children [1–3]. Milk and milk products are the most diversified natural foodstuffs, which contain more than 20 different minor and trace elements. Most of them like Cu, Zn, Mn and Cr, are essential and very important for normal metabolism, growth and development [4,5]. As a consequence of their nutritional and toxicological relevance, determination of the trace element in milk is of major importance. They fluctuate mainly according to physiological functions and extrinsic factors, as human activities, environmental and geological background, and others [4–8].

Inorganic mass spectrometry with inductively coupled plasma (ICPMS) is one of the most powerful analytical techniques for trace elemental determination due to its sensitivity, selectivity and multielemental and isotopic nature [9–12]. However, organic samples are challenging for conventional introduction systems of ICPMS, generating instability in the ICP, and even blockage in the interface co3nes. Milk is complex sample composed of a fat emulsion, casein micelle suspension and aqueous phase [13,14]. As a consequence, elemental analysis of milk samples by direct nebulization, even after water dilution, suffers from several inconveniences; i.e., deposition of organic matter in torch injector, spectral and non-spectral interferences, among others [6,15,16]. However, direct sample introduction into the plasma is achievable only with special sample introduction systems; i.e. replacing the conventional pneumatic nebulizer by a microconcentric one and a

cooled spray chamber, that produce a fine and stable dry aerosol at low flows [17]. Otherwise, introduction of digested and diluted samples is required in order to limit the amount of dissolved solids and organic matter [18–20]. This fact makes sample preparation one of the most relevant and decisive stages in the whole analytical procedure in the context of the trace elements analysis [19,21,22]. Recent contributions in this field propose soft wet treatment of milk samples, using acid (e.g. formic) or alkaline (tetramethylammonium hydroxide) agents [23–25] N,N-dimethylformamide (DMF) has been widely used as solvent in organic synthesis, and has also been utilized in chromatographic applications [26]. It has also been assayed in preparation of slurries for analysis of major, minor and trace elements in coal [27,28], and pharmaceutical samples by inductively coupled plasma optical emission spectrometry [29]. However, there are no antecedents of using DMF in sample dissolution for trace metal analysis.

Multielement data obtained by ICPMS for milk characterization is multivariate in nature [30]. These data comprise a list or array of values, called of first-order data, that could be used to extract relevant information from unsupervised and supervised chemometric techniques, such as principal component analysis (PCA), cluster analysis (CA), linear discriminant analysis (LDA), partial least square discriminant analysis (PLS-DA), among many others. PCA is an unsupervised multivariate method that offers information that enables discrimination of one particular sample from others [31,32].

In this work, a single-step sample preparation procedure is described for multielemental analysis of milk samples. DMF was used as organic diluent to simplify sample preparation and thus reducing significantly time of analysis. The coupling of a high-efficiency sample introduction system with a micronebulizer and a cooled spray chamber with ICPMS was investigated for direct introduction and analysis of DMF solutions. The method was successfully applied to real milk samples and a certified reference material was also analyzed for accuracy evaluation. Moreover, principal components analysis (PCA) was used to correlate the content of trace metals with the type of milk.

2. Material and methods

2.1. Instrumentation

An inductively coupled plasma mass spectrometer from Perkin-Elmer SCIEX, ELAN DRC-e (Thornhill, Canada) was used. The Ar gas with a minimum purity of 99.996% was supplied by Air Liquide (Río IV°-Córdoba, Argentina). An HF-resistant and high performance perfluoracetate (PFA) nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain lines, cooled with the PC^3 system from ESI (Omaha-NE, USA) were used.

Tygon black/black 0.76 mm i.d. and 40 cm length peristaltic pump tubing was used. The instrument conditions were: auto lens mode, peak hopping measurement mode, dwell time of 15 ms, 30 sweeps per reading, 1 reading per replicate and 3 replicates. Nickel sampler and skimmer cones were used. Before changing the pneumatic to the microconcentric nebulizer, a performance check for sensitivity and oxide and doubly charged ion formation, using a conventional cross flow nebulizer and a Scott spray chamber was carried out. For comparison purposes, the samples were digested in a microwave digestion system model START-D and hermetically sealed 100-mL internal volume, 1-cm wall thickness polytetrafluoroethylene (PTFE) reactors from Milestone (Sorisole, Italy), using a two steps program (10 min up to 200 °C and up to 1000 W and kept by 20 min at 200 °C, up to 1000 W). The digested samples were analyzed by ICPMS using the conventional nebulizer (cross-flow) and spray chamber (Scott type) and external calibration with

[103]Rh as internal standard. A water bath model HH-S from Arcano (Argentina) was used for sample pre-treatment with DMF.

2.2. Reagents and samples

The used water was distilled and de-ionized, with a resistivity of 18.2 MΩ cm, produced by an Easy pure RF system from Barnstead (Dubuque, IA, USA). Concentrated nitric acid (65% w/w) from Sigma-Aldrich (Germany), methanol from Fisher-Scientific (Lawn, NJ, USA), hydrogen peroxide (40% w/w) from Parafarm (Argentine), tetramethylammonium hydroxide pentahydrate from Sigma-Aldrich (USA), Dimethylformamide from Acroorganics (New Jersey, USA), formic acid (98% w/w) from Fisher Scientific (UK), were used throughout. Certified multi-element standard solutions 2, 3 and 5, and Rh mono-elemental standard solution from Perkin Elmer Pure Plus-Atomic Spectroscopy Standards, (Norwalk, USA) were used for calibration and recovery studies. The analyzed samples were commercially available milk samples of different brands from Argentina. A certified reference material namely, Skim milk powder (BCR 063R) from Community Bureau of reference, Geel, Belgium was employed for method validation.

2.3. Analytical procedure

As an alternative of extensive pretreatment before measurement, such as digestion with mineral acids and oxidants at elevated temperatures, we propose a sample treatment with DMF at room temperature. This sample preparation protocol is simple and fast, and also potential volatile analyte losses are prevented.

For element quantification, samples were prepared as follows: approximately 0.5 g of milk was accurately weighed in a 15 mL polypropylene flask and then, 2 mL of DMF were added and shaken vigorously. The volume was completed to 10 mL with HNO$_3$ (1.0%). The certified reference material was prepared just as the samples. After the optimization process, the selected conditions shown in Table 1 were set for all further determinations: the solutions were introduced into the plasma source at 700 μL min^{-1} applying 1100 W RF power and 0.7 L min^{-1} nebulizer gas flow rate.

Parallel microwave enhanced acid treatments were carried out for comparative purpose. For the digestion procedure, 0.5 g of milk samples were mixed with 7.0 mL of HNO$_3$ and 1.0 mL of H$_2$O$_2$ in PTFE flasks, then they were submitted to an optimized MW temperature program and after its competition diluted to 50 mL.

Two alternative strategies were evaluated for calibration of ICPMS, i.e. external calibration with aqueous standards (acidified

Table 1
Experimental setup and instrumental operating conditions for the analysis of milk by ICP-MS after solubilization with DMF.

| **Perkin-Elmer SCIEX, ELAN DRC-e (Thornhill, Canada)**	
Sample uptake rate (μL min^{-1})	700
Sample introduction	Nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain line, cooled with the PC3 system from ESI (Omaha – NE, USA)
Radiofrequency power (W)	1100 W
Gas flow rates (L min^{-1})	Plasma, 13.5; auxiliary, 1.2; nebulizer, 0.7
Sampler cone	Ni, i.d. 1.1 mm
Skimmer cone	Ni, i.d. 0.9 mm
Scanning mode	Peak hopping
Dwell time (ms)	30 in standard mode
Number of replicates	3
Sample treatment	0.5 g of milk+2 mL of DMF+agitation, up to 10 mL with HNO$_3$ (1.0%)
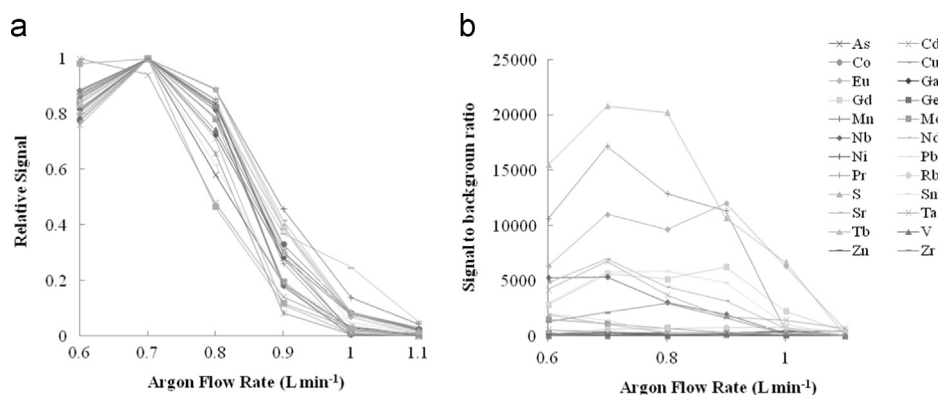
Fig. 1. (a) Ar flow rate at −5° C for the sample pretreated with DMF. (b) Signal-to-background ratio vs Ar flow rate for a DMF solution.
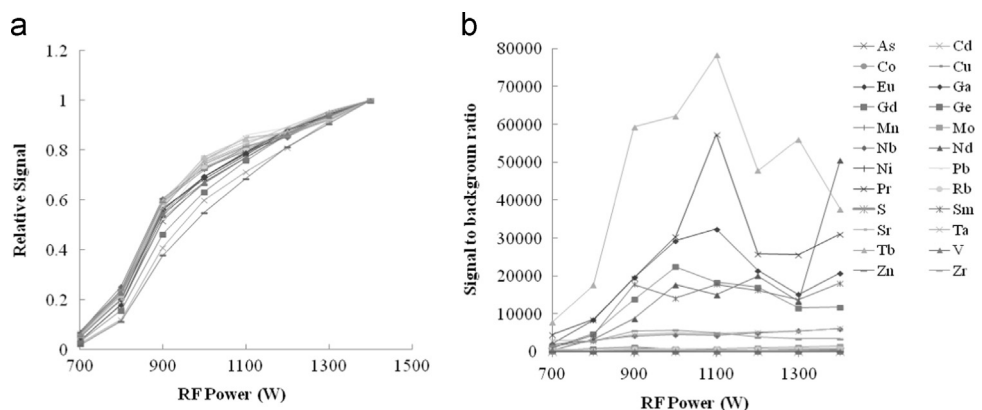

Fig. 2. (a) Radiofrequency power effect upon analyte signals and (b) upon signal-to-background ratio in a DMF solution.
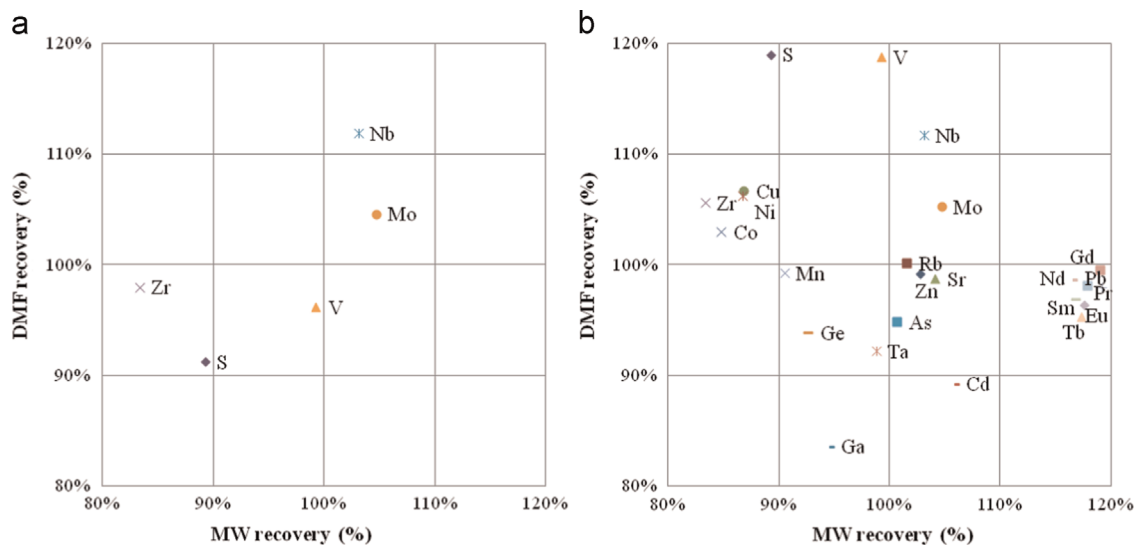

Fig. 3. Correlation graphs between the proposed method and microwave treatment, with (a) aqueous calibration and, (b) matrix matching calibration with DMF. Internal standard: [103]Rh.

with 1.0% nitric acid), and matrix matching calibration (added with 20%v/v DMF and 1% nitric acid). The analyte concentrations were 1; 5; 10; 20; 40 and 80 μg L^{-1}. Rhodium (10 μg L^{-1}) was added to all solutions as internal standard.

2.4. Principal component analysis

Principal component analysis (PCA) was performed as a descriptive tool to visualize the data in two dimensions using the statistical software package Unscrambler 6.11 (CAMO-ASA, Trondheim, Norway).

PCA is an unsupervised technique that reduces the dimensionality of the original data matrix retaining the maximum amount of variability [32]. This method provides new variables as linear combinations of the original variables, which are called principal components, allowing the visualization of samples in a new dimensional space and identifying the directions in which most of the information is retained [30,31]. It allows explaining relationships among variables and type of milk from data sets and assessing those variables that mostly contribute to the classification of samples.

3. Results and discussion

3.1. Instrument optimization

Organic compounds could improve not only the ionization rate of elements with high ionization energy by carbon charge transfer reactions, but also can raise the nebulization and aerosol transport rates, resulting in signals enhancement [18,19,33]. However, carbon ensuing from solvents and milk matrix may generate depositions on cones and lenses with consequent signal drifts and losses in sensitivity. These issues may be overcome by following an overall instrument optimization. To this aim, a solution containing only the reagents (procedural blanks) was compared in order to evaluate the background contribution of some carbon-containing polyatomic ions. Sample introduction was carried out using a PFA high-efficiency microconcentric nebulizer coupled to a baffled cyclonic spray chamber, at $-5\,°C$ as desolvating temperature (Section 2.1). The argon gas flow (Ar FR) and radiofrequency power (RF power) were optimized for maximum analyte intensity and minimum background. This was done through evaluation of the measured analytical signals in terms of ArFR (mL min^{-1}) and RF power (watts) obtained with both synthetic sample and a reagent blank (Figs. 1 and 2).

In Fig. 1, the effect of Ar flow rate (nebulizer) is shown. The optimum conditions were selected according to the relative raise of analytes signals (Fig. 1a) compared to the background generated due to matrix compounds (Fig. 1b). The best condition found was 0.7 L min^{-1} Ar flow rate, in which carbon-containing polyatomic ions did not contribute significantly, evidencing that efficient elimination of the solvent in the spray chamber was achieved. In Fig. 2a, the analyte signals vs applied RF power was plotted, while Fig. 2b shows the contribution to background of carbon-based polyatomic ions. The outcome indicates that despite better sensitivities were reached at 1400 W, the strong background contribution (lower SBR) shifted the optimum to 1100 W RF power as a compromise solution.

Finally, sample flow rate (SFR) was optimized to reach the best nebulization efficiency and also, to handle sample (matrix) input to the ICP. A characteristic SFR plot was thus obtained, where all signals increased up to a sample flow rate of 0.7–0.8 mL min^{-1} and remained almost constant at higher rates. Consequently, 0.7 mL min^{-1} SFR was

Table 3
Means and standard deviations for the determined analytes in Skim milk powder, BCR 063R ($n=3$).

Analytes	Certified values [µg g^{-1}]	Confidence limits[a]	Experimental values [µg g^{-1}]	Confidence limits[a]
As			0.020	0.003
Cd			< LD	–
Co			0.067	0.028
Cu	0.602	0.019	0.600	0.052
Eu			< LD	–
Ga			< LD	–
Gd			< LD	–
Ge			< LD	–
Mn			0.224	0.008
Mo			0.287	0.052
Nb			< LD	–
Nd			< LD	–
Ni			1.561	0.296
Pb	0.0185	0.0027	0.0144	0.0037
Pr			< LD	–
Rb			17.981	0.837
Sm			< LD	–
S[b]			47.627	2.429
Sr			3.729	0.200
Ta			< LD	–
Tb			< LD	–
V			0.037	0.002
Zn	49.0	0.6	45.9	2.54
Zr			< LD	–

[a] $\frac{t\,(0.05:2)*S}{\sqrt{n}}$.

[b] Determined as $^{32}S^{16}O^{+}$.

Table 2
Instrumental limit-of-detection for the trace elements determination in milk samples treated with DMF.

Analite	Isotope (u.m.a.)	LOD[a] [µg L^{-1}]	RSD [20 µg L^{-1}]
As	75	1.34	4.70
Cd	111	1.44	4.30
Co	59	1.08	6.60
Cu	63	1.04	2.90
Eu	153	0.84	3.60
Ga	69	2.64	6.20
Gd	158	0.64	1.30
Ge	74	2.48	3.30
Mn	55	1.80	2.90
Mo	98	7.72	0.50
Nb	93	0.80	1.80
Nd	142	1.16	4.80
Ni	60	4.16	7.20
Pb	208	0.80	4.60
Pr	141	1.04	2.90
Rb	85	0.92	3.40
Sm	152	0.88	2.90
S[b]	48	26.20	2.40
Sr	88	1.56	1.50
Ta	181	1.04	3.70
Tb	159	0.68	1.00
V	51	1.48	2.10
Zn	66	30.76	7.40
Zr	90	0.76	5.10

[a] Instrumental limit of detection.
[b] Measured as $^{32}S^{16}O^{+}$.

Table 4
Comparison between concentration values found using the proposed method and the microwave acid digestion.

Analite	Proposed method (DMF treatment) [µg L^{-1}]	Conventional method (microwave digestión) [µg L^{-1}]
As	< LD	< LD
Cd	< LD	< LD
Co	2.16 ± 0.17	1.83 ± 0.14
Cu	367.1 ± 7.6	318.82 ± 6.61
Eu	< LD	< LD
Ga	< LD	< LD
Gd	0.74 ± 0.17	0.87 ± 0.2
Ge	< LD	< LD
Mn	194.2 ± 1.5	175.7 ± 1.3
Mo	21.1 ± 1.1	22.1 ± 1.2
Nb	1.7 ± 0.4	1.9 ± 0.4
Nd	< LD	< LD
Ni	26.1 ± 0.9	22.6 ± 0.8
Pb	7.4 ± 1.1	8.8 ± 1.3
Pr	< LD	< LD
Rb	176.8 ± 7.4	179.6 ± 7.5
Sm	< LD	< LD
S[b]	1099 ± 51	1283 ± 60
Sr	403.4 ± 24.5	420 ± 25.5
Ta	< LD	< LD
Tb	< LD	< LD
V	4.6 ± 0.6	4.9 ± 0.6
Zn	5295 ± 6	5441 ± 6
Zr	< LD	< LD

Table 5
Results obtained in the analysis of milk available in Argentina.

Analyte [µg L⁻¹]	A1 Baby 1 0–6 months	A2 Baby 2 6–12 months	A3 Baby 3 1–3 years	AP1 Baby Premium 1 0–6 months	AP2 Baby Premium 2 6–12 months	AP3 Baby Premium 3 1–3 years	AP Baby Plus 3 1–3 years	AW Whole milk Adults
As	< LD	< LD	2.58 ± 0.51	< LD	1.44 ± 0.14	1.44 ± 0.03	< LD	2.80 ± 0.03
Co	2.16 ± 0.17	2.74 ± 0.31	3.88 ± 0.57	3.94 ± 0.93	2.92 ± 0.01	3.76 ± 0.34	5.58 ± 0.54	6.90 ± 0.36
Cu	367.1 ± 7.6	517.7 ± 2.5	27.1 ± 0.7	400.8 ± 1.3	515.5 ± 19.4	26.58 ± 0.48	21.22 ± 0.31	31.64 ± 0.39
Eu	< LD	< LD	< LD	1.34 ± 0.19	< LD	< LD	< LD	< LD
Gd	0.74 ± 0.17	< LD	< LD	0.96 ± 0.25	< LD	< LD	1.18 ± 0.01	0.9 ± 0.1
Mn	194.2 ± 1.47	59.8 ± 1.5	37.1 ± 0.2	219.3 ± 7.8	55.0 ± 2.4	39.12 ± 0.28	38.94 ± 4.21	27.86 ± 1.89
Mo	21.14 ± 1.13	17.8 ± 3.6	31.5 ± 0.1	12.38 ± 0.79	11.6 ± 0.02	16.38 ± 0.84	12.18 ± 0.51	27.6 ± 0.31
Nb	1.74 ± 0.42	< LD	< LD	< LD	< LD	< LD	3.40 ± 0.39	< LD
Nd	< LD	< LD	1.28 ± 0.1	2.12 ± 0.08	< LD	< LD	1.12 ± 0.08	< LD
Ni	26.12 ± 0.96	54.6 ± 1.8	61.9 ± 1.9	66.26 ± 11.62	55.9 ± 0.9	70.8 ± 1.1	129.8 ± 4.1	157.7 ± 0.25
Pb	7.36 ± 1.05	< LD	< LD	< LD	0.98 ± 0.17	< LD	< LD	< LD
Pr	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
Rb	176.82 ± 7.38	449.2 ± 23.4	478.9 ± 4.3	124.0 ± 2.8	387.7 ± 5.6	478.6 ± 16.3	319.5 ± 3.4	555.0 ± 13.04
Sm	< LD	< LD	< LD	1.36 ± 0.06	< LD	< LD	1.18 ± 0.02	1.24 ± 0.11
S	1099 ± 51	2852 ± 97	3159 ± 123	1112 ± 15	2605 ± 5	3246 ± 134	2498 ± 158	3194 ± 47
Sr	403.4 ± 24.5	1077 ± 42	1316 ± 51	377.7 ± 3.2	939 ± 19	1343 ± 49	1047 ± 1	720.4 ± 6.0
Tb	< LD	< LD	< LD	0.84 ± 0.14	< LD	< LD	0.88 ± 0.03	< LD
V	4.96 ± 0.65	6.26 ± 0.85	7.48 ± 0.31	18.04 ± 1.89	5.84 ± 0.08	6.36 ± 0.36	6.86 ± 0.57	17.08 ± 0.37
Zn	5295 ± 6	5838 ± 162	10101 ± 61	7888 ± 375	8569 ± 300	10148 ± 217	7907 ± 227	2815 ± 30

Analyte [µg L⁻¹]	B1 Baby 1 0–6 months	B2 Baby 2 6–12 months	B3 Baby 3 1–3 years	BW Whole milk adults	BWRL Whole milk Lactose Reduced adults	CW Powder milk kids	CWL Light powder milk kids	DW Whole milk adults
As	2.86 ± 0.45	1.82 ± 0.22	< LD	< LD	3.22 ± 0.19	3.83 ± 0.09	2.92 ± 0.44	< LD
Co	5.72 ± 0.45	7.96 ± 0.91	7.92 ± 0.06	7.1 ± 0.03	5.53 ± 0.34	4.3 ± 0.09	5.92 ± 0.24	5.86 ± 0.19
Cu	391.3 ± 10.6	573.8 ± 20.9	219.2 ± 4.5	31.88 ± 3.17	25.5 ± 0.44	18.2 ± 0.3	29.33 ± 0.37	25.16 ± 0.22
Eu	< LD	0.88 ± 0.17	< LD	< LD	< LD	< LD	< LD	< LD
Gd	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
Mn	967.4 ± 17.5	708.1 ± 41.4	469.0 ± 20.5	28.8 ± 7.4	17.07 ± 0.49	17.3 ± 0.1	38.23 ± 1.62	23.26 ± 2.91
Mo	7.60 ± 1.50	11.48 ± 1.44	20.16 ± 6.64	24.0 ± 6.9	33.50 ± 0.08	32.16 ± 0.12	27.6 ± 0.8	18.1 ± 0.9
Nb	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
Nd	< LD	< LD	1.28 ± 0.37	< LD	< LD	< LD	< LD	< LD
Ni	54.74 ± 5.34	123.1 ± 7.38	153.1 ± 11.7	162.5 ± 28.7	121.9 ± 6.8	101.1 ± 2.2	131.6 ± 5.6	141.5 ± 7.5
Pb	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
Pr	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
Rb	387.3 ± 13.5	354.2 ± 22.3	602.9 ± 22.7	691.8 ± 72.1	697.8 ± 14.4	433.7 ± 0.6	497.5 ± 10.5	437.8 ± 8.0
Sm	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
S	1967 ± 40	2260.2 ± 90.6	2973 ± 161	3288 ± 413	3230 ± 139	2374 ± 22	3181 ± 42	2881 ± 132
Sr	415.6 ± 15.0	588.2 ± 30.5	647.9 ± 35.6	757.2 ± 86.8	761.5 ± 30.1	562.4 ± 9.7	749.3 ± 14.8	958.7 ± 23.8
Tb	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD
V	9.0 ± 0.6	12.1 ± 1.9	11.16 ± 0.71	13.44 ± 3.31	11.30 ± 0.31	11.12 ± 0.67	8.80 ± 0.22	10.1 ± 0.5
Zn	5129 ± 95	4282 ± 132	4251 ± 232	2981 ± 229	2621 ± 119	3994 ± 68	5158 ± 23	2293 ± 117.7

thus established for all further experiments aiming to minimize the amount of carbon to be introduced to the ICP.

3.2. Calibration strategy: matrix effects

With the aim to evaluate and minimize matrix effects and to come across to a proper calibration strategy for accurate analysis of milk samples treated with DMF, two approaches were evaluated; i.e. aqueous (Fig. 3a) and matrix matching (Fig. 3b) calibration. Thus, milk samples were prepared as recommended in Section 2.3, spiked with 10 µg L^{-1} of the analytes and the obtained accuracies (as % recoveries) were compared (Fig. 3) with those obtained after microwave digestion.

The recoveries of the proposed method when different approaches for calibration were used were paired with the recoveries obtained in spiked samples submitted to microwave digestions (Fig. 3). This study allowed finding differences or errors due to matrix effects mainly, but also as consequence of bad sample handling. Fig. 3a shows that in the case of external calibration with aqueous standards, only Mo, Nb, S, V and Zr were accurately measured (recoveries between 80% and 120%). On the other hand, Fig. 3b shows that matrix matching calibration is needed to ensure quantitative determination of most analytes is achievable, if compared with microwave digestion.

3.3. Analytical performance

The recommended procedure involved low dilution factors (0.5 g of sample diluted up to 10 mL with water after treatment with 2 mL of DMF) resulting in low detection limits (DL) for most analytes which were evaluated as 3.3 times the standard deviation ($n=10$) of the blank. The precision was calculated as percent relative standard deviation (RSD %, $n=3$) for a standard with a concentration at the middle of the calibration plot. It is important to highlight that according to the complexity of samples being analyzed, the analytical performance (Table 2) was satisfactory compared with some previous works [14,15].

Skim milk powder (BCR 063R) from Community Bureau of Reference was employed for accuracy check. The certified values and the found values for the analytes Cu, Pb and Zn that are present in the reference material are shown in Table 3. The results were compared through a paired *t*-test and no significant differences were observed ($n=3$, $p=0.05$). Other analytes that were not informed were also determined. A comparison between concentration values found using the proposed method vs the conventional one (microwave digestion) in one sample, is shown in

table 4. Spiked aliquots of this sample were also evaluated and the quantitatives recoveries could be observed in Fig. 3.

3.4. Analytical validation and application

Milk samples (labeled as: A, B, C and D according to their type and trade marks) are shown in Table 5. Each sample was independently treated with DMF as recommended and analyzed in triplicate. The average concentrations of the analytes found in each milk sample are shown in Table 5.

In all cases the concentrations of Cd, Ga, Ge, Pr, and Ta were lower than their respective detection limits. The presence of As, Co, Cu, Eu, Gd, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, S, Sm, Sr, Tb, V and Zn was evidenced in one or more milk samples. In the case of As, Cd and Pb, they complied with the maximum limits established by the Codex Alimentarius Commission, being <0.05; <0.05; <0.02 mg kg^{-1} respectively.

3.5. Principal components analysis

The principal components analysis model was applied to the matrix formed by the multielemental concentrations corresponding to milk samples. Thus, a matrix of 32×24 was built from the autoscaled data, without prior signal pretreatment. The PCA model was built using 6 variables, corresponding to Cu, Mn, Rb, S, Sr and Zn concentrations (Table 5). Fig. 4a shows the score plot of the first two PCs using selected variables. The first two PC's used to build the model, explained more than 98% of the total variance in the data set of milk sample analyzed. PCA graph shows the formation of four groups. From the loadings plots (Fig. 4b) it was determined that the most outstanding variables in the discrimination of the groups were S, Zn, and to a lesser extent, Sr, Rb, Mn and Cu. The samples separation through the first component is correlated with the Zn low, medium and high concentration in samples from whole milks (adults) (I), baby milks (II) and baby milks fortified (premium or plus) (III), respectively. There is also the formation of a subset of the second component corresponding to baby milks administered in the first few months of life (IV), which require larger amounts of Mn and Cu and less S. It is noticeably that sample A3, unfortified milk, fell within fortified milk group. Additionally, A3 showed an identical classification to sample AP3. Perhaps these samples are very similar because they belong to the same factory and they are the same sample in different packaging. However, deeper insights cannot be reached with PCA and instead, discriminant analysis (DA) must be used with a larger sampling size.
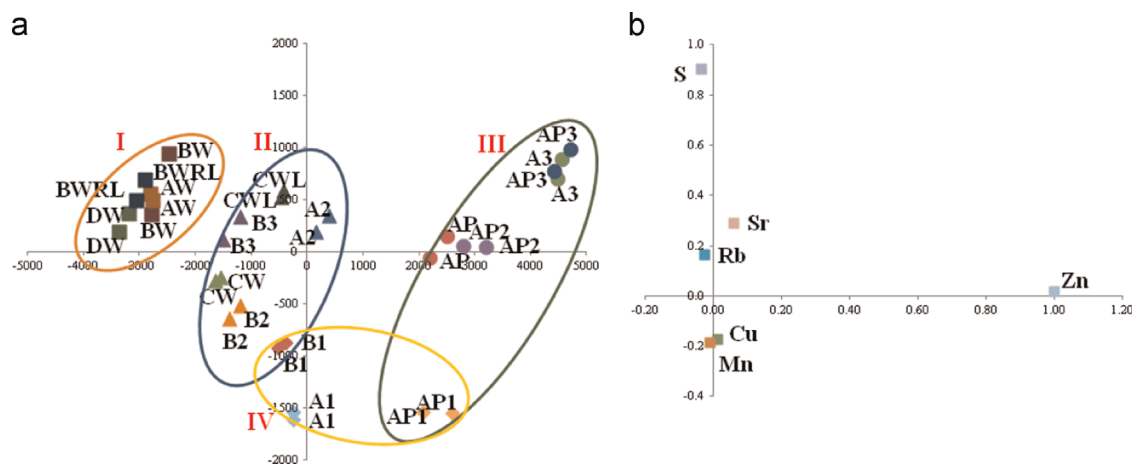

Fig. 4. (a) PCA scores plot for the first two PCs. (b) Loadings plot for PC1 and PC2.

4. Conclusion

Samples of milk are suitably solubilized with DMF, enabling their introduction to the ICPMS using a microconcentric nebulizer operated at $-5\,^\circ\text{C}$. This undemanding and rapid sample preparation procedure makes the proposed method an attractive approach for routine analysis considering mainly the multielement analysis capabilities of complex matrices with high carbon concentration, as milk. Furthermore, the proposed method is fast, simple, precise, accurate and less expensive than other approaches. In addition, the achieved sensitivities are higher than those reached with conventional microwave digestion. The quantification by external matrix matching calibration with DMF, using ^{103}Rh as internal standard, was recommended for As, Cd, Co, Cu, Eu, Ga, Gd, Ge, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sm, S, Sr, Ta, Tb, V, Zn, and Zr determination. A reference material, skim milk powder (BCR 063R), and also enriched samples were analyzed to check accuracy, yielding quantitative recoveries. PCA studies have been performed with six variables: Zn, S, Mn, Cu, Rb and Sr. Valuable information could be obtained allowing milk classification (adult, baby or baby fortified) according to elemental contents. Results from this study emphasized the importance of the multielement determination of trace elements in milk samples to carry out traceability and/or origin denomination studies and also to assess their quality.

Acknowledgments

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