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Analytical methodology

Validation of a dilute and shoot method for quantification of 12 elements by inductively coupled plasma tandem mass spectrometry in human milk and in cow milk preparations

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ABSTRACT

Nutritional information about human milk is essential as early human growth and development have been closely linked to the status and requirements of several macro- and micro-elements. However, methods addressing whole mineral profiling in human milk have been scarce due in part to their technical complexities to accurately and simultaneously measure the concentration of micro- and macro-trace elements in low volume of human milk.

In the present study, a single laboratory validation has been performed using a “dilute and shoot” approach for the quantification of sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo) and iodine (I), in both human milk and milk preparations.

Performances in terms of limits of detection and quantification, of repeatability, reproducibility and trueness have been assessed and verified using various reference or certified materials. For certified human milk sample (NIST 1953), recoveries obtained for reference or spiked values are ranged from 93% to 108% (except for Mn at 151%).

This robust method using new technology ICP-MS/MS without high pressure digestion is adapted to both routinely and rapidly analyze human milk micro-sample (i.e. less than 250 µL) in the frame of clinical trials but also to be extended to the mineral profiling of milk preparations like infant formula and adult nutritionals.

1. Introduction

Characterization of minerals and their content determination in biofluids and tissues are crucial given that essential mineral macro-nutrients and trace elements play important structural and biological roles regarding human metabolism, growth and development [1]. Over the last decades, numerous analyses of minerals and trace elements have been realized in human milk [2–5]. It is especially important to determine the mineral composition of breastfeeding as human milk is the biofluid recommended as the ideal and reference or exclusive source of nutrition for term and preterm infants in their first months of life [6]. As a result, generation of reliable data and reference levels of minerals in human milk is mandatory for public health authorities and pediatric professionals to establish reliable dietary recommendations for intake of inorganic nutrients during infancy and childhood [7,8]. These nutritional information are hence essential for food industry to mimic the mineral composition of human milk through the development of adequate fortified infant formula products when breastfeeding may not be always possible or suitable [9–11]. For this reason, due to fortification, infant formula and adult nutritional products have higher content of some minerals than human milk.

Trace element composition in human milk varies due to many factors including stage and type of lactation, mother’s genetics and health status, maternal diet, body store, environmental conditions and cultural habits of lactating women [12–15]. Robust and reliable methods addressing whole mineral profiling in human milk have been often scarce due in part to their technical complexities to accurately and simultaneously measure elemental concentrations in a generally small available amount of test sample.

Compared to other traditional analytical atomic spectrometric techniques historically used like atomic absorption (AAS) or inductively coupled plasma mass optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS) is recognized today as the best tool to simultaneously quantify elements in biological fluids with precision and low limits of quantification [16–22]. Both single quadrupole (Q-ICP-MS) and high resolution (HR-ICP-MS)

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equipment have been specifically used for the determination of trace and ultra-trace elements in human milk in the two last decades [23,24]. The recent commercial launch of an ICP-tandem mass spectrometer (ICP-MS/MS) using specific gas in collision / reaction cells (CRC) have significantly improved the management of isobaric interferences and the possibility to determine simultaneously major and trace elements in complex matrices [25,26]. However, the preparation procedure for a low volume of human milk sample amenable to ICP-MS analysis is critical as minimal handling and time consumption are needed for a clinical study requiring high throughput routine analysis. Compared to a conventional complete acid digestion using microwave systems [27–31], simple alkaline dilution [32] or alkaline extraction [33] is nowadays preferred since good analytical performances were obtained not only for human milk and milk powders [33,34] but also for other biological samples [35–37]. A recent study using alkaline dilution of human milk micro-sample (i.e. 0.2 mL) has shown the successful simultaneous analysis of eight essential trace elements by ICP-MS [24].

In the present work, a full single laboratory validation using a similar preparation approach was performed for the determination of twelve essential major and trace elements by ICP-MS/MS in both human milk micro-samples (i.e. less than 250 µL of sample) and processed cow milk products including milk powders, infant formula and adult nutritional products.

This simple dilute and shoot routine ICP-MS method is demonstrated to be fit for purpose according to ISO 17025 norm [38] for the rapid and high-throughput determination of sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo) and iodine (I), in both human milk and processed cow milk products. This robust method is also validated to be routinely applied for analysis of these elements in human milk during future clinical trials.

2. Experimental

2.1. Reagents and standards

Ultrapure water (H_2O), (18.2 MΩ cm^{-1}) obtained from a Milli-Q purification device (Millipore, Bedford, MA, USA) was used throughout this work for the preparation of samples and solutions. Human milk and milk powder samples are diluted with an alkaline solution prepared from ammonia solution (NH_4OH) 25% suprapur to solubilize cells and stop protein precipitation, 2-propanol p.a. quality purchased from Merck (Merck, Darmstadt, Germany) used as carbon buffer, ethylene diamine tetra-acetic acid EDTA trace metals basis 99.995% to stabilize metals in basic solution and Triton X-100 Bioxtra from Aldrich (Aldrich, St Louis, Missouri, USA) to improve ICP-MS sample introduction. Internal standards for elemental quantification (tellurium (Te) and germanium (Ge)) were prepared from Merck solutions 1000 mg/L, ICP grade (Merck, Darmstadt, Germany). Finally, a custom- blend multi elemental stock solution containing 11 elements (K, Na at 500 mg/L, Ca, P at 500 mg/L, Mg at 100 mg/L, Zn at 5 mg/L, Fe, Cu at 1 mg/L, Mo at 0.2 mg/L, Se at 0.1 mg/L and Mn at 0.01 mg/L) and an iodine solution at 0.5 mg/L were purchased from Labkings (Labkings, Hilversum, Netherlands).

Preparation of solutions and samples were all performed in laminar flow fume hoods.

Two alkaline dilution solutions were prepared in polypropylene bottles to dilute blank, standards, milk powders, ready to feed and human milk samples.

The first solution (dedicated to prepare method blank, standards, milk powders and ready to feed products) was firstly prepared by mixing 100 mL 2-propanol (corresponding to final concentration 10% v/v), 10 g EDTA (corresponding to final concentration 1% v/v), 5 mL Triton X-100 aqueous solution 1% v/v (corresponding to final concentration 0.005% v/v), 100 mL ammonia solution (NH_4OH) 25% suprapur (corresponding to final concentration 10% v/v), 1000 µL Germanium ICP grade solution 1000 mg/L, 1000 µL Tellurium ICP grade solution 1000 mg/L and adjusted to 1 L with ultra-pure water. The solution was then thoroughly mixed by shaking and finally put into an ultrasonic bath to reduce dissolved gases forming bubbles.

The second solution (dedicated to human milk samples) was prepared by mixing 50 mL of the first solution and 430 mL ultra-pure water.

An ammonia solution 5% v/v was prepared by mixing 10 mL ammonia solution (NH_4OH) 25% suprapur with 40 mL ultra-pure water.

Standards were prepared mixing 1 mL first alkaline dilution solution with diluted custom-blend multi elemental stock solutions and ultra-pure water adjusted to 10 mL in 15 mL volumetric polypropylene tubes giving a final concentration of 2-Propanol 1% v/v, EDTA 0.1% v/v, Triton X 100 surfactant 0.0005% v/v and NH_4OH 1% v/v.

One blank standard (Std1) and 5 standards (i.e. Std2 to Std6) were prepared in order to cover the concentration range of all elements in all matrices : Na from 2 to 100 mg/L, Mg from 0.2 to 10 mg/L, P from 1 to 50 mg/L, K from 2 to 100 mg/L, Ca from 1 to 50 mg/L, Cu from 2 to 100 mg/L, Fe from 2 to 100 mg/L, K from 2 to 100 mg/L, Mg from 0.2 to 10 mg/L, Mn from 0.02 to 1 µg/L, Na from 2 to 100 mg/L, P from 1 to 50 mg/L, Zn from 10 to 500 µg/L, Se from 0.2 to 10 µg/L, I from 1 to 50 mg/L, Mo from 0.4 to 20 µg/L.

An additional level of Mn concentration was used to cover the full range of concentration found in samples.

2.2. Sample preparation

Human milk samples were simply prepared mixing 0.2 mL human milk with 4.8 mL alkaline dilution solution.

Milk powders, infant formula and adult nutritional powdered products were firstly reconstituted weighing 200 mg powder, then mixed and vortexed with 8.8 g of ultrapure water and finally 1 g of ammonia solution 5% was added. A second dilution was realized (with a factor ranging from 5 to 20 according to sample concentration) using the alkaline dilution solution.

Only the second dilution was performed for ready to feed (RTF) products.

Method blank samples were prepared mixing 0.4 mL ultrapure water with 9.6 mL alkaline dilution solution.

All analyses were performed directly after sample preparation.

2.3. Instrument parameters

A triple quadrupole ICP-MS/MS (Agilent™ 8800 model, Agilent, Santa Clara, USA) was used for the full validation. Instrument daily tune was realized according to manufacturer's recommendations. Instrumental parameters are presented in Table 1.

2.4. Samples

Six following reference or certified human milk and milk powder samples were used to assess precision, trueness and uncertainty of the method:

- Human milk: LEE BIOSOLUTIONS, cat. N° 991-01-P, pooled donors (no reference value). Milks were collected after more than four weeks after giving birth.
- Human milk: NIST SRM-1953 (no certified value for Zn, Se, Mo and I according to January 2016 certificate). Milk coming from six milk banks located around the US (Florida 4%, North Carolina 6%, Iowa 6%, Delaware 7%, California 12% and Texas 65%) was collected and pooled.
- Commercial infant formula (IF1)
- Skimmed milk powder: European Reference Material (ERM®)-BD150 (certified spiked values for Cu and Fe)
- Non-fat milk powder: NIST SRM-1549