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# Determination of Microelements in Human Milk and Infant Formula Without Digestion by ICP-OES

**Dijana Đurović,[1,*] Branka Milisavljević,[2] Mirjana Nedović-Vuković,[1] Branislav Potkonjak,[3] Snežana Spasić[3] and Miroslav M. Vrvić[4]**

[1] *Institute of Public Health of Montenegro, Džona Džeksona bb, 81000 Podgorica, Montenegro,*

[2] *Department of Neonatology, Subotica Hospital, Izvorska 3, 24000 Subotica, Serbia,*

[3] *Department of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 1, Belgrade, Serbia,*

[4] *Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade, Serbia*

** Corresponding author: E-mail: dil@t-com.me*
*Tel/Fax ++ 382 (0) 20 412 888*

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## Abstract

The concentrations of zinc (Zn), iron (Fe) and copper (Cu) in both human milk and infant formula were determined using a new sample preparation method, by inductively coupled plasma – optical emission spectometry (ICP-OES) and flame atomic absorption spectrometry (FAAS). Human milk samples were diluted in ultrapure water. The infant formula of powder samples (suitable for an infant 1–6 months of age) and standard reference material (SRM-1849) were analyzed in parallel**.**

The results have shown that FAAS method was more sensitive for Fe determination in human milk while ICP-OES was more sensitive for both Zn and Cu detection. The limit of quantification for both Zn and Cu was 5 μg $L^{-1}$ and 10 μg $L^{-1}$ for Fe and the recovery for Zn, Fe and Cu was ranged from 90% to 94%, 97% to 103% and 90% to 102%, respectively. Mean concentrations of Zn, Fe, and Cu in human milk samples were 5.35, 0.47 and 0.83 mg $L^{-1}$, respectively while these values in infant formula were ranged from 3.52–4.75 mg $L^{-1}$, 3.37–4.56 mg $L^{-1}$ and 0.28–0.41 mg $L^{-1}$, respectively. Despite the sample complexity, the proposed method using dilution of milk samples with water was simple, rapid, effective and accurate. ICP-OES was a better method for Zn determination while FAAS was a better method for Fe determination. In the case of Cu both methods were comparable.

**Keywords:** Microelements, human milk, method validation, sample preparation

## 1. In troduction

Human breast milk is a complex mixture of nutrients. The World Health Organization recommends breast milk as the ideal food for the growth of infants under 12 months of age.[1] Human milk contains almost all the essential components (minerals, vitamins, essential amino and fatty acids) required for normal growth and development of newborns.[2–4] Microelements are important constituents of a large number of molecules including structural proteins and catalytic enzymes. Zinc (Zn), iron (Fe), and copper (Cu) are essential microelements for the normal growth of an infant. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) proposed standards for infant formula composition, specifically 0.5–1.5 mg of Zn and 0.3–1.3 mg of Fe in 100 kcal of infant formula.[5] Human milk during early childhood (the first few months of a baby´s life), provides both protection for the immune system and healthy development.[6] Zn is an essential microelement for many biochemical processes, especially for infants as it is a cofactor and constituent of almost 300 enzymes. Zn participates in nucleic acid metabolism, synthesis and turnover of proteins, lipids, carbohydrates, cell replication and gene regulation.[7] During pregnancy, childhood and adolescence Zn supports healthy development and normal

growth.[8–10] Copper (Cu) is another very important microelement for enzyme catalysis crucial for balanced metabolism.[11] Moreover, Cu regulate myelin sheath production in the nervous system, melanin synthesis and normal thyroid gland function.[12] Cu has both, antioxidant and pro-oxidant properties. Another trace element of interest is iron (Fe) which is absolutely required for hemoglobin synthesis and red blood cell formation and for regulation of oxidative reactions.[9–14] Human milk demonstrates some bacteriostatic properties most likely due to the content and/or bioavailability of Fe in breast milk. Many *in vivo* and *in vitro* studies have confirmed that Fe is a key factor in breast milk to protect against pediatric pathogens.[15] Therefore, a deficiency or excess of these microelements could threaten enzyme activities and biological processes in the human body.[16,17]

Definition of nutritional requirements as well as physiology of milk secretion for infants is based on appropriate date of micronutrient content in human milk during lactation. Determination of trace elements content is very important since the examined matrix is very complex emulsion, with low metal ions concentration. Previous studies using a number of different methods have been published.[18–20] Atomic absorption spectrometry was one of the first techniques employed and it is still used in clinical and dairy product analysis.[21] ICP-OES, FAAS, electrothermal atomic absorption spectrometry (EAAS) and inductively coupled plasma – mass spectometry (ICP-MS) are standard techniques nowadays.[22,23] A new method for Cu determination, zeeman electrothermal atomic absorption spectrophotometry (ZEAAS), was recently established. It does not require sample digestion, requires minimal preparation and uses two chemical modifiers.[24] Determination by inductively coupled plasma – atomic emission spectroscopy (ICP-AES) and ICP-MS of major and minor elements has used milk dilution procedures with 5 or 10% v/v water-soluble extract mixed with tertiary amine reagent at pH 8.[25]

Despite the fact that ICP-MS is highly sensitive, accurate and precise, it is the least used technique in clinical laboratories due to high instrument cost and demanding protocols.[26–28] Neutron activation analysis (NAA) is one of the most sensitive techniques for applications in clinical biology. However, analyses are time consuming and therefore largely inappropriate.[29]

Microwave-assisted digestion is the most common sample preparation method for clinical and diary samples.[30] Despite the fact that the technique is fast and simple, strong acids for sample degradation are needed. There is a lack of information regarding a unified human sample preparation procedure. Accordingly, the first aim of our study was to introduce a new method for human milk and infant formula sample preparation based on simple water-based dilution without chemical consumption. This could be considered as an environmentally-friendly "green method" for clinical and diary sample preparation. This is similar to inorganic ion determination using minimal nitric acid consumption and water dilution in donkey´s milk by ion chromatography.[31]

The second aim of this study was to optimize microelement analysis using ICP-OES via an easy, rapid, cost-effective and environmentally acceptable procedure amenable to most laboratories.

# 2. Material and Methods

## 2. 1. Sample Collection and Storage

Twenty-eight human milk samples (10–20 ml) were obtained from healthy mothers (aged 31.2 ± 6 years) on a first day after on-term delivery at the Department of Neonatology in Subotica Hospital in January 2013. All the mothers gave written consent for milk sampling. The Ethical Committee of the Faculty of Medicine in Belgrade approved the study (No.01-434/4, dated 22/05/2012). The study protocol adhered to general guidelines laid down by the Ethical Committee of the Faculty of Medicine.

Every mother cleaned her breast and nipple with ultrapure water (Milli-Q, conductivity <1μS/cm) using protective gloves prior to milk sampling using a manual breast milk pump and/or a passive breast milk sampler. The women were instructed to sample milk both at the beginning and at the end of the breast-feeding session. Milk samples were collected in clean plastic vessels which were previously washed with ultrapure water. Human milk samples were labeled and stored at –20°C before analysis. Random samples of infant formula were obtained from the local market (five different manufacturers) in Montenegro (APTAMIL 1, BEBELAC 1, HIPP, NAN 1 and IMPAMIL®MIL1). IMPAMIL®MIL1 was produced in Serbia. Others originated from EU countries. Formulae were purchased in triplicate from January to April, 2013. The samples were stored in a dark and dry location until analysis, which was performed before the expiry date.

Human milk samples were defrosted at room temperature and diluted 10 times with ultrapure water. Infant formulae samples were prepared as follows: 1 g was made up to 100 ml of ultrapure water, due to the higher concentrations of the microelements and higher density of the resulting solutions in comparison to diluted human milk samples. This procedure allowed analyses to fall within the linear ranges of the analytical standard calibration curves. Our methodology allowed direct analysis of samples without digestion and took into consideration possible interference from organic matter during the detection of the microelements**.** Using the above-mentioned dilution procedures, the samples were prepared in such a way to be very similar (fat and protein content) and enabled us to correctly validate the method. The content of the analyzed microelements in the samples was similar to the chosen reference material.

## 2. 2. Reagents

All chemicals used were purchased as analytical grade. Zn, Fe and Cu analytical solutions were prepared after serial dilutions of stock reference solutions containing 1000 mg $L^{-1}$ of each element (LGC-ICP-OES stock solution). Reference material from the National Institute of Standards and Technology (NIST), and infant/adult nutritional formula SRM-1849 were used as calibrators.

## 2. 3. Equipment

The ICP-OES instrument (with axial configuration) was purchased from Spectro Analytical Instruments GmbH (Kleve, Germany). It was controlled using Smart Analyzer Vision Software (version 5.01.0928) and connected to an ASX-520 auto sampler (CETAC). Spectro ICAL solution (10x concentrate, Berd Kraft Der Standard) was used for self-checking and self-adjustment of the instrument (over the entire polychromator). ICP-OES instrument parameter settings are shown in Table 1.

**Table 1**. ICP-OES instrument parameter settings

| Parameter | Setting |
|---|---|
| Plasma Power | 1.4 KW |
| Pump Speed | 30 Rpm |
| Coolant Flow | 14 L $min^{-1}$ |
| Auxiliary Flow | 0.7 L $min^{-1}$ |
| Nebulizer Flow | 0.9 L $min^{-1}$ |
| Spray chamber | Cyclonic |
| Plasma viewing mode | Axial |
| Processing mode | Area |
| Metal (wavelength, nm) | Zn (213.857), Fe (238.204), Cu (327.396) |
| Correlation Coefficient | 0.999 |
| Number of replicates | 3 |
| Rinse delay | 30 s |
| Read delay | 30 s |

## 2. 4. Statistical Analysis

Statistical analyses were conducted using SPSS Version 17.0 for Windows. Correlation coefficients for Zn, Fe and Cu in human milk samples were established (Pearson coefficient). The results were considered to be statistically significant if $p$ was < 0.05. Linear regression was used to evaluate the differences between FAAS and ICP-OES. Descriptive statistics was used for data evaluation. The results are presented as tables and box plots. The boxes represent the median and the 25$^{th}$ and 75$^{th}$ percentiles; the whiskers represent the non-outlier range. Outliers and extremes were defined as data point values that were more than 1.5× and 3× the interquartile range (IQR) outside of the box.

# 3. Results and Discussion

## 3. 1. Method validation

Table 2 summarizes sensitivity and linearity. The linearity of the measurement was assessed by analyzing five standard solutions prepared by diluting the standard mixture solution (1000 mg $L^{-1}$) in ultrapure water. All standards (0, 0.05, 0.1, 0.5 and 1.0 mg $L^{-1}$) were measured in triplicate. Standards were analyzed on a regular basis for the purpose of monitoring instrument drift. In order to monitor cross-contamination and sample loss blank solutions were analyzed on a regular basis.[32] Surface area *vs.* metal ion concentration was plotted and calibration curves were constructed. The aim of the study was to obtain a correlation coefficient of $R^2 > 0.999$ for linearity.[32] Since the correlation coefficients for Zn, Fe and Cu were $R^2 > 0.9999$, the aim of study was met. Within the measurement range deviations from theoretical values did not exceed 5% which demonstrated good correlation between element concentration and surface area. To evaluate sensitivity of the analytical method the limits of detection (LOD) and limits of quantification (LOQ) were used. Their values were determined as (3 σ/S) and (10 σ/S), respectively**.** According to International Union of Pure and Applied Chemistry (IUPAC) recommendations, detection and quantification limits were calculated where σ was the standard deviation (SD) of the response to ten calibration blanks and S was the slope of the analytical curve. The highest detection limit was for Fe, 3.0 mg $L^{-1}$. Precision was evaluated through the determination of the coefficient of variation (CV) by measuring the relative standard deviation.[33] For each sample duplicate measurements were repeated as six replicates per day for intra-assay variation. The standard deviations (SDs) of intra-assay variation were 4% for Zn, 8% for Fe and 1% for Cu by ICP-OES and 3% for Zn, 3% for Fe and 0.6% for Cu by FAAS. Coefficients of variation (CVs) were acceptable for clinical sample detection (CV < 7%). CV values were lower from FAAS, whereas the recovery of Zn and Cu was superior from ICP-OES.

**Table 2**. Analytical method validation

| Element | Relative precision (%) | $R^2$ | Linearity range (μg $L^{-1}$) | LOD (μg $L^{-1}$) | LOQ (μg $L^{-1}$) |
|---|---|---|---|---|---|
| Zn | 4 | 0.99994 | 0.9–2400 | 1.5 | 5 |
| Fe | 8 | 0.99971 | 0.7–2400 | 1.5 | 5 |
| Cu | 1 | 0.99957 | 1.2–2400 | 3.0 | 10 |

### 3. 1. 2. Accuracy

There was no suitable and available reference material for human milk. Accordingly, the method accuracy was assessed using the SRM. Quality control was verified by recovery experiments for the three selected microelements. Recoveries of the analyzed microelements by ICP-OES and FAAS for SRM NIST-1849 are presented in Table 3. Although milk is a complex matrix composed of proteins, carbohydrates and lipids accuracy indicated good recovery from two different microelement spiked concentrations (10 and 50 μg $L^{-1}$) in human milk pools (Table 4).[28] Specifically, average recovery ranged from 90% to 94% for Zn, 90% to 102% for Cu by ICP-OES and 97% to 103% for Fe by FAAS (Table 4). Blank samples and the 0.25 mg $L^{-1}$ control standard were evaluated together with samples in each batch (Table 5).

**Table 3.** Recovery (%) of NIST 1849 by ICP-OES and FAAS

| Element | ICP-OES | FAAS |
|---|---|---|
| Zn | 91 | 85 |
| Fe | 93 | 100 |
| Cu | 99 | 98 |

**Table 4.** Recoveries of spiked (10 and 50 μg $L^{-1}$) Zn, Fe and Cu in milk samples from lactating mothers

| Element | Min % | Max % |
|---|---|---|
| Zn | 90 | 94 |
| Fe | 97 | 103 |
| Cu | 90 | 102 |

**Table 5.** Recovery and precision for control standard 0.25 mg $L^{-1}$

| Element | Mean ± SD | Recovery (%) | RSD (%) |
|---|---|---|---|
| Zn | 0.2632 ± 0.0085 | 105.3 | 3.40 |
| Fe | 0.2592 ± 0.0043 | 103.7 | 1.65 |
| Cu | 0.2583 ± 0.0032 | 103.3 | 1.23 |

ICP-OES and FAAS analysis of Zn, Fe and Cu in SRM-1849 infant/adult Nutritional Formula and infant formula (Aptamil, Bebelac, NAN1, HIPP and Impamil Mil1) are summarized in Tables 6 and 7. The concentrations of Zn, Fe and Cu in SRM-1849 obtained by FAAS and ICP-OES were similar with the stated reference values. Better results for Zn and Cu were obtained by ICP-OES whereas FAAS provided a better result for Fe. For each sample, duplicate tests were repeated 6 times per day for intra-assay comparison. Analytical characteristics (linearity, sensitivity, precision and accuracy) were considered satisfactory for analysis of clinical and infant formula samples by ICP-OES.

### 3. 1. 3. Clinical Sample Detection

After method validation 28 samples of human milk were analyzed for Zn and Cu content by ICP-OES while Fe content was measured by FAAS. Results of human milk analysis are shown in Table 8, while concentration correlation with dietary reference intake of Institute of Medicine[34] between Zn, Fe and Cu is to be found in Table 9.

The results reported in Table 8 showed that Zn concentration on a first day after delivery (5.35 ± 2.15

**Table 6.** Detected (by ICP-OES) and certified and labeled values of microelements in infant formula and SRM. Minimum and maximum values are expressed as mean values (n = 12)

| | | Detected values mg/100g of powder | | | Certified and labeled values |
|---|---|---|---|---|---|
| | | Min | Max | Mean ± SD | mg/100g of powder |
| Zn | SRM | 12.89 | 14.25 | **13.93 ± 0.4** | 15.23 ± 0.5 |
| | Aptamil 1 | 3.49 | 3.71 | **3.52 ± 0.2** | 3.6 |
| | Bebelac 1 | 3.28 | 3.55 | **3.48 ± 0.3** | 3.6 |
| | HIPP | 3.75 | 4.10 | **3.86 ± 0.4** | 4.0 |
| | NAN 1 | 4.65 | 5.10 | **4.75 ± 0.4** | 5.4 |
| | Impamil®Mil1 | 4.25 | 4.85 | **4.45 ± 0.2** | 4.3 |
| Fe | SRM | 13.88 | 17.04 | **16.47 ± 0.8** | 17.71 ± 0.3 |
| | Aptamil 1 | 3.59 | 3.94 | **3.78 ± 0.4** | 3.9 |
| | Bebelac 1 | 3.38 | 3.71 | **3.63 ± 0.3** | 3.9 |
| | HIPP | 3.73 | 4.21 | **3.89 ± 0.5** | 4.0 |
| | NAN 1 | 4.45 | 4.95 | **4.56 ± 0.4** | 5.2 |
| | Impamil®Mil1 | 3.26 | 4.00 | **3.37 ± 0.1** | 3.6 |
| Cu | SRM | 1.83 | 2.07 | **2.01 ± 0.10** | 2.03 ± 0.04 |
| | Aptamil 1 | 0.26 | 0.33 | **0.30 ± 0.05** | 0.29 |
| | Bebelac 1 | 0.26 | 0.31 | **0.28 ± 0.03** | 0.29 |
| | HIPP | 0.25 | 0.28 | **0.26 ± 0.05** | 0.27 |
| | NAN 1 | 0.34 | 0.41 | **0.38 ± 0.05** | 0.40 |
| | Impamil®Mil1 | 0.30 | 0.36 | **0.32 ± 0.06** | 0.36 |

**Table 7.** Detected (by FAAS) and certified and labeled values of microelements in infant formula and SRM. Minimum and maximum values are expressed as mean values (n = 12)

| | | Detected values mg/100g of powder | | | Certified and labeled values |
|---|---|---|---|---|---|
| | | Min | Max | Mean ± SD | mg/100g of powder |
| Zn | SRM | 12.74 | 13.14 | **12.90 ± 0.3** | 15.23 ± 0.5 |
| | Aptamil 1 | 2.75 | 3.21 | **3.10 ± 0.4** | 3.6 |
| | Bebelac 1 | 2.85 | 3.15 | **3.09 ± 0.4** | 3.6 |
| | HIPP | 3.37 | 3.75 | **3.58 ± 0.3** | 4.0 |
| | NAN 1 | 4.12 | 5.00 | **4.55 ± 0.5** | 5.4 |
| | Impamil®Mil1 | 3.89 | 4.15 | **4.01 ± 0.1** | 4.3 |
| Fe | SRM | 17.23 | 18.30 | **17.74 ± 0.3** | 17.71 ± 0.3 |
| | Aptamil 1 | 3.55 | 3.85 | **3.61 ± 0.3** | 3.9 |
| | Bebelac 1 | 3.67 | 3.90 | **3.75 ± 0.3** | 3.9 |
| | HIPP | 3.78 | 4.10 | **3.95 ± 0.3** | 4.0 |
| | NAN 1 | 4.98 | 5.29 | **5.05 ± 0.5** | 5.2 |
| | Impamil®Mil1 | 3.25 | 3.75 | **3.55 ± 0.1** | 3.6 |
| Cu | SRM | 1.82 | 2.21 | **1.99 ± 0.06** | 2.03 ± 0.04 |
| | Aptamil 1 | 0.27 | 0.32 | **0.29 ± 0.03** | 0.29 |
| | Bebelac 1 | 0.25 | 0.30 | **0.27 ± 0.03** | 0.29 |
| | HIPP | 0.21 | 0.27 | **0.24 ± 0.04** | 0.27 |
| | NAN 1 | 0.34 | 0.40 | **0.37 ± 0.02** | 0.40 |
| | Impamil®Mil1 | 0.31 | 0.37 | **0.33 ± 0.08** | 0.36 |

mg $L^{-1}$) was higher than in the previously reported study.[35.] Mean values for Zn in a study reported by Honda were very similar to our study and it was 5.32 mg $L^{-1}$.[36] Based on this data Zn concentration on a first day after delivery are in accordance to the Daily Recommended Intake (DRI) and can satisfied infant needs. The mean concentration of Zn reported for donkey milk, that is considered as a best replacement for human milk, reported in some studies are comparable to our human milk study.[23,37,38.]

The mean Fe concentration determined for clinical samples was 0.83 ± 0.99 mg $L^{-1}$ and as such it was lower than the value reported in the previous studies as well as in some studies for donkey milk.[23,35,37,39] The mean concentration of Cu in human milk samples was 0.47 ± 0.20 mg $L^{-1}$**.** The obtained data pointed out that Fe content showed the same trend as Zn, which is a lower concentration in both, human and donkey milk as it has been established in the previous studies.[37,39] It could be concluded that on a first day of delivery level of Zn, Fe and Cu human milk is sufficient to meet the recommended values.

There is no statistically significant correlation between Zn, Fe and Cu content in human milk samples ($p > 0.05$).

The obtained data showed that ICP-OES method provided statistically significantly higher average values

**Table 8.** Descriptive analysis of for Zn, Fe and Cu in human milk samples

| Element | Concentration, (mg $L^{-1}$) | | | |
|---|---|---|---|---|
| | Min. | Max. | Mean ± SD | DRI (mg) |
| Zn | 2.22 | 9.91 | 5.35 ± 2.15 | 2.00 |
| Fe | 0.32 | 5.50 | 0.83 ± 0.99 | 0.27 |
| Cu | 0.12 | 0.92 | 0.47 ± 0.20 | 0.20 |
| N | | | 28 | |

**Table 9.** Correlation concentration coefficient for Zn, Fe and Cu in human milk samples

| Element | Cu | Zn | Fe |
|---|---|---|---|
| Cu | 1 | | |
| Zn | –0.135 | 1 | |
| Fe | 0.116 | –0.069 | 1 |

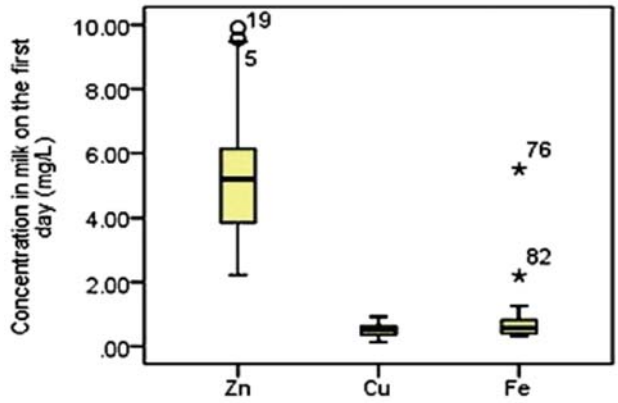


**Figure 1.** Concentration of Zn, Fe and Cu in human milk samples on a first day after the delivery. The boxes represent the median and the 25th and 75th percentiles; the whiskers represent the non-outlier range.

for 9.5 mg $L^{-1}$ for Zn when compared to FAAS ($p = 0.001$). Method FAAS provided statistically significantly higher average values of 23.25 mg $L^{-1}$ for Fe when compared to ICP-OES ($p < 0.001$). For Cu, there was no statistically significant difference between both methods ($p = 0.671$). Figure 1 shows changes of Zn, Cu and Fe concentration in human milk samples on the first day after the delivery.

## 4. Conclusion

In this study, a fast, easy, economical and simple method for sample preparation was developed (no degradation) in order to determine the essential trace elements in human breast milk. Method evaluation and clinical sample detection on ICP-OES were used for validation of accuracy, reliability and practicality. The main advantage of presented sample preparation method is the fact that there is no chemical consumption and it is considered to be a green chemical method, i.e. it is environmentally acceptable. In conclusion, the proposed method is simple, economical, accurate, and highly reliable and as such, it can be applied to the clinical detection of trace elements in biological samples. There is no statistically significant correlation between Zn, Fe and Cu concentrations in human milk samples. Based on the obtained data and linear regression, it could be concluded that ICP-OES is a better method for Zn determination, FAAS for Fe, while there is no statistically significant difference between these two methods, when Cu is concerned.

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**Conflict of Interest:** All authors declare no conflict of interest.

**Ethical approval:** "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

**Informed consent:** Informed consent was obtained from all mothers that donated milk samples.

## 6. References

1. R. Mahdavi, L. Nikniaz, B. P. Gargari, S. G. Hezaveh, *Iran. Biol. Trace. Elem. Res.* **2010**, *135*, 174–181 https://doi.org/10.1007/s12011-009-8510-y
2. K. Ljung, B. Palm, M. Grandér, M. Vahter, *Food Chem.* **2011,** *127*, 943–951. https://doi.org/10.1016/j.foodchem.2011.01.062
3. M. L. Cross, H. S. Gill, Br. J. Nutr. **2000**, *84*, 81–89. https://doi.org/10.1017/S0007114500002294
4. B. Lonnerdal, B. *Nutrition.* **2000**, *16*, 509–511.
5. B. Koletzko, S. Baker, G. N. Cleghorn, F. Ulysses, S. Gopalan, O. Hernell, Q. S. Hock, P. Jirapinyo, B. Lonnerdal, P. Pencharz, H. Pzyrembel, J. Ramirez-Mayans, R. Shamir, D. Turck, Y. Yamashiro, D. Zong-Yi, *J. Pediatr. Gastroenterol. Nut.r* **2005**, *41*, 584 –599. https://doi.org/10.1097/01.mpg.0000187817.38836.42
6. O. T. Oftedal, *Animal.* **2012**, *6*, 355–368. https://doi.org/10.1017/S1751731111001935
7. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA)s Scientific Opinion on *the* substantiation of health claims related to zinc. EFSA **2009,** J7 (9): 122:34.
8. R. Gibson, A.-L. Heath, *Nutr. Diet*. **2011,** *68*, 97–108. https://doi.org/10.1111/j.1747-0080.2011.01516.x
9. J. Bryan, S. Osendarp, D. Hughes, E. Calvaresi, K. Baghurst, J.-W. Van Klinken, *Nutr. Rev*. **2004**, *62*, 295–306. https://doi.org/10.1111/j.1753-4887.2004.tb00055.x
10. W. Maret, H. H. Sandstead,. *J. Trace. Elem. Med. Biol.* **2006**, *20*, 3–18. https://doi.org/10.1016/j.jtemb.2006.01.006
11. E. D. Harris, *Nutr. Rev.* **2001,** *59*, 281–285. https://doi.org/10.1111/j.1753-4887.2001.tb07017.x
12. J. Osredkar, N. Sustar, *J. Clin. Toxicol.* **2011,** S3-001.
13. B. Lozoff, M. K. Georgieff, *Semin. Pediatr. Neurol.* **2006,** *13*, 158–165. https://doi.org/10.1016/j.spen.2006.08.004
14. F. M. Rioux, J. Bélanger-Plourde, C. P. Leblanc, F. Vigneau, *Can. J. Diet. Pract. Res*. **2011**, *72*, e140-e146.
15. J. G. Dorea, *Nutrition.* **2000**, *16*, 209–20. https://doi.org/10.1016/S0899-9007(99)00287-7
16. J. Koolman, K. H. Roehm, Color Atlas of Biochemistry.2nd Edition, Thieme **2005**, pp 362–363.
17. M. Hannan, B. Faraji, J. Tanguma, N. Longoria, R. C. M. Rodriguez, *Biol. Trace. Elem. Res.* **2009**, 127, 6–15. https://doi.org/10.1007/s12011-008-8221-9
18. D. Silvestre, M. J. Lagarda, R. Farré, C. Martìnez-Costa, J. Brines. *Food Chem.* **2000**, *68*, 95–99. https://doi.org/10.1016/S0308-8146(99)00160-0
19. R. Melø, K. Gellein, L. Evje, T. Syversen, *Food Chem. Toxicol*. **2008**, *46*,3339–42. https://doi.org/10.1016/j.fct.2008.08.007
20. K. Ljung Björklund, M. Vahter, B. Palm, M. Grandér, S. Lignell, M. Berglund, *Environ Heal.* **2012,** *11*, 92. https://doi.org/10.1186/1476-069X-11-92
21. Y. Luo, B. Zhang, M. Chen, J. Wang, X. Zhang, W. Y. Gao, J. F. Huang, W. L. Fu, *J. Agric. Food. Chem.* **2010**, *58*, 9396–9400. https://doi.org/10.1021/jf100940e
22. R. S. Nascimento, R. E. S. Froes, N. O. C. Silva, R. L. P. Naveira, D. B. C. Mendes, W. B. Neto, J. B. B. Silva, At. Spectrosc. 2010, 43, 960–971.
23. A. G. Potortì, G. Di Bella, V. Lo Turco, R. R., R. Rando, G. Dugo, J. Food Compos. Ana. **2013**, *31,* 161–172. https://doi.org/10.1016/j.jfca.2013.05.006
24. A. Pineau, B. Fauconneau, A. Marrauld, A. Lebeau, R. Han-

kard, O. Guillard, *Biol. Trace. Elem. Res.* **2015**, *166*, 119–122. https://doi.org/10.1007/s12011-015-0249-z
25. J. A. Nobrega, Y.Gelinas, A. Krushevska, R. M. Barnes, *J. Anal. At. Spectrom.* **1997**, *12*, 1243–1246. https://doi.org/10.1039/A607492H
26. W. Wasowicz, J. Gromadzinska, K. Szram, K. Rydzynski, J. Cieslak, Z. Pietrzak, *Biol. Trace. Elem. Res*. **2001**, *79*, 221–33. https://doi.org/10.1385/BTER:79:3:221
27. M. Krachler, T. Prohaska, G. Koellensperger, E. Rossipal, G. Stingeder, *Biol. Trace. Elem. Res.* **2000**, *76*, 97–112. https://doi.org/10.1385/BTER:76:2:97
28. Khan N, Jeong IS, Hwang IM, J. S. Kim, S. H. Choi, E. Y. Nho, J. Y.Cho, K. S. Park, K. S. Kim, (2014) *Food Chem*. **2014**, *147*, 220–224. https://doi.org/10.1016/j.foodchem.2013.09.147
29. M. Hannan, N. N. Dogadkin, I. Ashur I, W. M. Markus, *Biol. Trace. Elem. Res.* **2005**, *107*, 11–20. https://doi.org/10.1385/BTER:107:1:011
30. M. Yaman, N. Çokol, *At. Spectrosc*. **2004**, *25*, 185–190.
31. G. Di Bella, V. Lo Turco, A. G. Potortì, R. R. Luppino, V. Fotia, F. Conte, G. Dugo, *Food Addit. Contam*. *A*. **2012**, *29*, 1021–1029. https://doi.org/10.1080/19440049.2012.674979
32. N. Khan, I. S. Jeong, I. M. Hwang, J. S. Kim, S. H. Choi, E. Y. Nho, J. Y. Choi, B.-M. Kwak, J.-H. Ahn, T. Yoon, K. S. Kim. *Food Chem*. **2013,***141*, 3566–3570. https://doi.org/10.1016/j.foodchem.2013.06.034
33. M.Chudzinska, D. Baralkiewicz, Foo*d Chem. Toxicol.* **2011**, *49*, 2741–2749. https://doi.org/10.1016/j.fct.2011.08.014
34. Institute of Medicine, Dietary Reference Intakes; the Essential Guide to Nutrient Requirements, National Academy Press, Washington DC, **2006**,
35. M. T. S. Andrade, L. A. Del Ciampo, I. R. Lopes Del Ciampo, I. S. Ferraz, Fernando Barbosa Junior, *Food Nutr Sci*. **2014**,1196–1201. https://doi.org/10.4236/fns.2014.513130
36. R. Honda, K. Tawara, M. Nishijo, H. Nakagawa, K. Tanebe, S. Saito, *Toxicology,* **2003**, *186*, 255–259. https://doi.org/10.1016/S0300-483X(03)00002-7
37. E. Salimei, F. Fantuz, Int. Dairy J. **2012**, 24, 146–152. https://doi.org/10.1016/j.idairyj.2011.11.008
38. V. A. Soares, M. M. M. Kus, A. L. C. Peixoto, J. S. Carrocci, R. F. S. Salazar, H. J. I. Filho, *Food Control.* **2010**, 21,45–49. https://doi.org/10.1016/j.foodcont.2009.03.010
39. N. Yamawaki, M. Yamada, T. Kan-no, T. Kojima, T. Kaneko, A. Yonekubo, J. Trace. Elem. Med. Biol. **2005,** *19*, 171–181. https://doi.org/10.1016/j.jtemb.2005.05.001

## Povzetek

Z uporabo optične emisijske spektrometrije z induktivno sklopljeno plazmo (ICP-OES) in plamenske atomske absorpcijske spektrometrije (FAAS) smo v vzorcih humanega mleka in vzorcih mlečnih formul za dojenčke določili koncentracije cinka, železa in bakra. Poleg mlečnih formul za dojenčke stare od 1 do 6 mesecev smo analizirali tudi standardni referenčni material SRM-1849. Za Fe smo najboljšo občutljivost določili s FAAS, za Zn in Cu pa z ICP-OES. Meja določanja za Zn in Cu je 5 μg $L^{-1}$ in 10 μg $L^{-1}$ za Fe. Izkoristki za Zn, Fe in Cu so v območjih od 90 % do 94 %, 97 % do 103 % in 90 % do 102 %. V vzorcih humanega mleka so bile povprečne koncentracije Zn, Fe in Cu 5,35, 0,47 in 0,83 mg $L^{-1}$, medtem ko so bile v mlečnih formulah za dojenčke v območjih od 3,52–4,75 mg $L^{-1}$, 3,37–4,56 mg $L^{-1}$ in 0,28–0,41 mg $L^{-1}$. Kljub kompleksnosti vzorcev je predlagana metoda preprosta, hitra in učinkovita. Za določanje Zn je boljša ICP-OES metoda, za določanje Fe pa FAAS. V primeru Cu sta metodi primerljivi.