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# Development and validation of an analytical method for the determination of arsenic, cadmium and lead content in powdered infant formula by means of quadrupole Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT

Cadmium (Cd), lead (Pb) and arsenic (As) can enter the food chain through the environment and/or as a consequence of the manufacturing process making foodstuffs the main human exposure route to these chemical elements. The risk associated with this exposure is of such a big concern for human health that the European Food Safety Agency recommends to reduce the exposure to Cd and Pb so as to protect especially vulnerable subgroups of population (*e.g.*, infants). Therefore, the setting of new maximum levels (MLs) for chemical elements in infant formulae (*e.g.*, for Cd) or the reconsideration of the existing ML for Pb is under discussion. On this basis, the availability of analytical methods, precise, accurate and sensitive enough to quantify low concentration values, is a key point especially for official control laboratories that have to state the sample compliance using a fully validated method with an associated uncertainty compliant with the requirements specified in the pertinent regulations. This work describes the development and validation of an analytical method to quantify As, Cd and Pb in powdered infant formulae based on animal protein at values of concentration close to the MLs that are likely to be set. The results obtained make the method suitable for a precise and accurate determination of these chemical elements at these low concentration values. In particular, the results for limit of quantification (LoQ) were respectively ($\mu g\ kg^{-1}$): As 6.2, Cd 1.2 and Pb 4.5. While for the recovery rates the following percentages were obtained: As 105%, Cd 98% and Pb 108%. The expanded uncertainties were found extremely satisfactory (Cd 13% and Pb 19%). The LoQ and the uncertainty for Pb meet the requirements set in Commission Regulation (EC) No. 333/2007 and following amendments being lower than the maximum values allowed. Even for Cd the expanded uncertainty resulted adequate in relation with the low concentration considered.



## 1. Introduction

The exposure to heavy metals is of such a big concern that numerous studies have been conducted to point out their potential hazards for human health (Sridhara Chary, Kamala, & Samuel Suman Raj, 2008; Wang, Sato, Xing, & Tao, 2005) and to assess the level of exposure (Han et al., 1998; Herreros, Iñigo-Nuñez, Sanchez-Perez, Encinas, & Gonzalez-Bulnes, 2008; Tressou, Crépet, Bertail, Feinberg, & Leblanc, 2004). Some publications called attention to the fact that many individuals in Europe exceed the TWI (Tolerable Weekly Intake) of certain metals (Cuadrado, Kumpulainen, & Moreiras, 1995; Nasreddine & Parent-Massin, 2002). As a consequence, the CONTAM Panel of the European Food Safety Agency recommended to reduce the exposure to both cadmium (Cd) and lead (Pb) (EFSA, 2009a; EFSA, 2010) and to produce data on the content of arsenic (As) in different food commodities as well (EFSA, 2009b). The health adverse effects of Cd, Pb and As have been thoroughly investigated (Buchet et al., 1990; Järup, 2003; Järup, Berglund, Elinder, Nordberg, & Vahter, 1998; Steenland & Boffetta, 2000) pointing out that infants and children are the most vulnerable groups, especially to Pb, due to the higher gastrointestinal uptake and less developed blood–brain barrier compared to adults. Thus, they can suffer damages even at the current levels of exposure to this heavy metal. As, Cd and Pb can

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enter the food chain through environmental pollution, accidental environmental contamination and/or as a consequence of manufacturing processes, pointing to foodstuffs as the main route of human exposure to these toxic elements. Infant feeding should be especially monitored taking into account both infant vulnerability and the fact that infant formulae (IFs) are the sole nutritional source in the first six months of life when breast-feeding is barred. The WHO Global Data Bank on Breastfeeding, which covers 94 countries and 65% of the world's infant population, estimated that only 35% of these infants are exclusively breast-fed in the first four months of life. The situation is different from country to country, but general data confirmed the large use of infant formulae both in the sole bottle feeding and in mixed feeding (combination of breast and bottle feeding). The most commonly used formulae are based on cow's milk and are conceived to be as similar as possible to breast milk from one to three months postpartum. However, a lot of differences exist in terms of content of essential elements due to the manufacturer's fortification (Ljung, Palm, Grandér, & Vahter, 2011; Rodríguez Rodríguez, Sanz Alaejos, & Díaz Romero, 2000). Therefore, a lot of works are available in literature, but most of them are focused on the determination of macroelements or oligoelements (Lesniewicz, Wroz, Wojcik, & Zyrnicki, 2010; Saracoglu, Saygi, & Uluozlu, 2007) present in IFs at high concentration also as a consequence of this enrichment. As for certain chemical elements (*e.g.* Cr, Ni, Cd, Pb) their level depends on the starting raw material (Tajkarimi et al., 2008) as well as on the possible contamination during the manufacturing process (i.e. release from containers used to prepare the sample). Due to the potential transfer of dangerous chemicals along the food chain, food safety is a key topic within the protection of public health. In fact, the foodstuffs marketed in the European Union have to be compliant with specific regulations in which maximum concentrations allowed are set as well. The control bodies (official laboratories, National Reference Laboratories) have to use methods of sampling and analysis whose requirements are established in ad hoc regulations. This means that the samples subjected to control have to be judged as compliant or not compliant based on the analytical results and taking into account the relevant uncertainties. As for chemical elements, maximum levels (MLs) for As, Cd, Hg and Sn (inorganic) in foodstuffs are set in Commission Regulation (EC) No. 1881/2006 and following amendment (CR No. 420/2011) while the requirements that analytical methods for official control have to fulfil are laid down in Commission Regulation (EC) No. 333/2007 amended by Commission Regulation (EU) No. 836/2011. In particular, the interpretation of results is included in the Point D of this last regulation prescribing that the analytical results have to be subtracted from the expanded measurement uncertainty and a sample can be considered as compliant only when the value obtained is lower than the relevant ML. This implies that the analytical methods have to be validated around the ML with an uncertainty lower than the maximum value allowed in CR No. 333/2007.

Although As and Cd are recognized as potentially toxic, no maximum levels (MLs) for these elements in milk and IF are set in the European legislation. As far as Pb is concerned, a ML equal to 0.020 mg $kg^{-1}$ is established for infant and follow-on formulae ready to use (liquid form or powdered reconstituted as instructed by manufacturer). A debate is in progress on the setting of a ML for Cd as well as on the possibility to apply the existing or a lower ML for Pb directly on powdered formula. Both these issues are mainly due to the widespread use of infant foods and the focus on human exposure to As, Cd and Pb. As a consequence, the development and the validation of analytical methods, sensitive, accurate and suitable to assess sample compliance around low values of concentration, is a key point especially for laboratories dealing with official food control. Furthermore, the methods used for official control should have comparable uncertainties so as to reach a uniform judgment on sample compliance within the European Union.

As for the analysis of Cd and Pb some works are based on data from techniques with a not adequate sensitivity when the values of concentration to be determined are quite low (Bakircioglu, Kurtulus, & Ucar, 2011; Ikem, Nwankwoala, Odueyungbo, Nyavor, & Egiebor, 2002; Kazi et al., 2010; Tripathi, Raghunath, Sastry, & Krishramoorthy, 1999), whilst few data on As are available. The aim of this work is to develop a method to quantify the content of As, Cd and Pb in powdered IFs using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and to validate this method at low concentration values of the analytes of interest. All the parameters (*e.g.* limit of quantification, accuracy, uncertainty) were set so as to have a procedure suitable for the assessment of sample compliance for Cd and Pb around low values of concentration close to the new MLs that are likely to be set. In particular, the analytical work was conducted in order to obtain an expanded uncertainty as low as possible. This implied to pay attention to each analytical phase (*e.g.* strict control of any source of contamination; occurrence of specific interferences) since IF is usually a matrix more complex than milk. In fact, although it is usually produced from cow's milk the necessity of making it as similar as possible to the breast milk accounts for its enrichment of nutrients and micro/macroelements.

Therefore, the occurrence of possible interferences from the elements with which the formulae are enriched was carefully taken into account. The method was completely validated for Cd and Pb and partially validated for As. The work was performed by the European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO), that is accredited both as testing laboratory according to ISO 17025 (ISO/IEC 17025, 2005) and as Proficiency Test (PT) Provider (Ciprotti, Sorbo, Orlandini, & Ciaralli, 2013; Sorbo, Colabucci, & Ciaralli, 2013) according to ISO 17043 (ISO/IEC 17043, 2010).

## 2. Materials and methods

### 2.1. Infant formula samples

The method was validated on IFs purchased in Italian supermarkets or pharmacies and based on animal protein. In particular, the formulae selected for validation purpose were those produced from cow milk collected within the European Union. A preliminary test batch spiked with Cd and Pb was prepared within the organization of a dedicated Proficiency Testing addressed to the National Reference Laboratories of EU Member States.

The same procedure was followed to obtain a test material spiked with As. Repeatability and reproducibility were assessed on the material coming from both the test batches, trueness was evaluated using the PT test items (17th PT) by comparison with the assigned values (for Cd and Pb) and by recovery rates (for As).

As far as the preparation of the test batches is concerned, the starting material was diluted, spiked and then lyophilised again. Several brands were checked in order to select the one that could be dissolved with the least quantity of water in order to avoid a long and expensive lyophilisation process.

The powdered IF was spiked with a standard solution containing the chemical elements of interest. The yield of the lyophilisation process was accounted for the preparation of the standard solutions so as to obtain the level of concentration that would be achieved in the final material. The starting powder, after reconstitution with the spiked solution was transferred into plastic boxes suitable for the freeze-drying process. The boxes were stored frozen until lyophilisation and the lyophilized material was mixed, quartered, bottled and labelled.