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Direct determination of lead in human milk by electrothermal atomic absorption spectrometry

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Abstract

Infants are particularly sensitive to the toxic effects of lead. Since milk is their only or main food it is important to know the contribution of milk to lead intake. The purpose of this study was to develop a direct method for determining the lead content of human milk by electrothermal atomic absorption spectrometry when a deuterium lamp is the only background correction available. The optimum conditions for lead determination in breast milk: sample dilution in Triton ×-100, modifier (75 μg of palladium) and nitric acid contents (2%) and the graphite furnace program (mineralization: 1100°C; atomization: 2300°C) were selected. The analytical parameters are: linearity (20–300 ng ml^{-1}); detection limit (5.0 ng ml^{-1}); precision (intra-assay 11.7%) and recovery percentage (109.8 ± 5.4%), and they show that the method is useful for determining lead in human milk.

1. Introduction

Lead is a common polluting element mainly because of its natural abundance and widespread industrial use. It is an antagonist of the essential elements Ca, Fe, I and probably Cu (Bryce-Smith, 1997). Infants are particularly sensitive to the toxic effects of lead. As milk is their only or main food it is important to know the contribution of milk to lead intake. Accumulation of lead during chronic exposure and its mobilization and secretion with mothers' milk constitute a serious health hazard for newborn infants (Lin-Fu, 1982; Mushak, 1991; Paci et al., 1991; Shannon and Graef, 1992). Toxic amounts of lead can lead to encephalopathy (Clarkson, 1987; Carton, 1988). A high percentage of the surviving children of exposed mothers suffer seizures and show signs of mental retardation. Anemia and reduced intelligence scores were observed in children after exposure to very low levels of lead (Clarkson, 1987; Carton, 1988; Mushak, 1991).

A wide range of lead contents in human milk: 7.6 μg l^{-1} (Ong et al., 1985); 0.5 μg l^{-1} (Hallen et al., 1995); 0.6 ng g^{-1} (Oskarsson et al., 1995); 0–472 μg l^{-1} (Guidi et al., 1992); 24 μg l^{-1} (Namihira et al., 1993) have been reported in the literature depending on geographical and environmental factors as well as on the methodology used for its determination (Schumann, 1990; Saleh et al., 1996; Frkovic et al., 1997).

The determination of metals in complex samples by atomic absorption spectrometry (AAS) generally requires the destruction of the sample matrix to obtain a solution of the analyte ready for analysis. For direct analysis of samples electrothermal AAS (EAAS) has been shown to offer several advantages over conventional wet oxidation and dry ashing sample preparation procedures. The advantages of direct determination are mainly a reduction in the sample preparation time; the possibility of analyte loss through volatilisation prior to analysis; the loss of analyte due to a retention by insoluble residues; and the possibility of a sample contamination. Several authors (Narres et al., 1985; Wagley et al., 1989; Larsen and Rasmussen, 1991; Hallen et al., 1995) have proposed methods for the direct determination of lead in milk by EAAS, but all of them use a Zeeman background correction.

The aim of this study is to develop a direct lead determination method for human milk by electrothermal atomic absorption spectrometry when a deuterium lamp is the only background correction available.

2. Methods and materials

2.1. Instrumentation

A Perkin–Elmer model 1100B spectrophotometer equipped with an HGA-graphite furnace atomizer was

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used. A Perkin–Elmer lead electrodeless discharge lamp with its own power supply (EDL system 2) was used.

2.2. Reagents

All reagents were of analytical reagent grade. Triton ×-100 (Panreac); nitric acid (sp.gr. 1.40 Merck); palladium 99.99% (Janssen Chimica); magnesium nitrate (Panreac); hydrochloric acid 37% (sp. gr. 1.19 Panreac); ammonium hydroxide 25% (sp. gr. 0.91).

Lead stock solution (Titrisol, Merck) 1000 mg l^{-1} in HNO_3 0.2%. Standard working solutions were prepared from stock solution immediately before use. Water was deionized (Milli-Q system, Millipore). All glassware was soaked in nitric acid for 10 min and rinsed with deionized water before use.

A palladium–magnesium nitrate mixture was used as matrix modifier. This mixture was prepared as follows: 150 mg of palladium was dissolved in 350 µl of HNO_3 and then 125 µl of HCl was added (solution A); 100 mg of $Mg(NO_3)_2$ was dissolved in 0.5 ml of deionized water (solution B). Both solutions were kept at least 12 h at room temperature before use and were then combined in 60 ml deionized water. The pH value was adjusted to 7.0 by adding NH_4OH and the solution was completed to 100 ml with deionized water.

2.3. Samples

A pool of human milk provided by volunteers (frozen immediately after the sampling) was collected.

2.4. Procedure

Samples of human milk were analysed directly by electrothermal atomic absorption spectrometry without preliminary treatment to destroy interfering organic compounds. In order to choose the optimum conditions for the determination of lead, different amounts of sample, Triton ×-100, nitric acid and modifier were assayed. The instrumental conditions applied are reported in Table 1.

Table 1
Instrumental conditions applied for lead determination

Wavelength (nm)	283.3
Slit width (nm)	0.7
Intensity lamp (mA)	380
Integration time (s)	5
Graphite tube	No pyrolitic
Gas used	Argon
Background correction	Deuterium lamp
Gas flow during atomization	Stop flow
Read	peak-height
Volume of sample (µl)	20

Table 2
Lead determination in breast milk: optimum conditions

100 µl standard (2% HNO_3) + 100 µl modifier (75 µg Pd) + 100 µl sample-Triton (3/2); volume injected: 20 µl

Graphite furnace program

Step	Temperature (°C)	Ramp (s)	Hold (s)
Drying	130	5	10
Charring	1100	5	15
Atomization	2300	0	5
Cleaning	2600	1	1

3. Results and discussion

3.1. Optimization of the analytical conditions

The graphite furnace program (temperature, heating ramp and hold time) were optimized in order to obtain the maximum integrated absorbance signals (see Table 2).

Different amounts of Triton ×-100, nitric acid, modifier and volume of sample were assayed to select the best conditions.

The dilution of the sample giving the best results was: 300 µl milk + 200 µl 0.25% Triton ×-100. This solution with the added lead standard was used to assay different amounts of nitric acid and modifier, always in the same proportions: 100 µl of diluted milk + 100 µl of standard (at different nitric acid concentrations) + 100 µl of modifier (at variable concentrations obtained by diluting the original).

The absorbance values corresponding to the injection of 20 µl of the different assayed combinations are represented in Fig. 1. The variables assayed were: (a) nitric acid (sp. gr. 1.40) content of the added lead standard (v/v): 4, 3, 2 and 1%. (b) Amount of modifier added: 100 µl (150 µg Pd), 75 µl + 25 µl H_2O (100 µg Pd), 50 µl + 50 µl H_2O (75 µg Pd).

The optimum conditions for lead determination in breast milk are reported in Table 2. Although the highest values of absorbance correspond to 4% nitric acid and 75 µg of palladium (mean absorbance values of ten replicates: 0.130 ± 0.016), 2% of nitric acid with the same amount of modifier was selected, because the variability of absorbance values was lower than when 4% nitric acid was used (mean absorbance values of 10 replicates: 0.120 ± 0.006).

3.2. Linearity, detection limit, precision and accuracy

To check the quality and usefulness of the direct lead determination in human milk by electrothermal atomic absorption spectrometry the analytical parameters were estimated.

3.2.1. Linearity

The linearity of the response was verified by spiking milk samples with different amounts of lead to achieve a

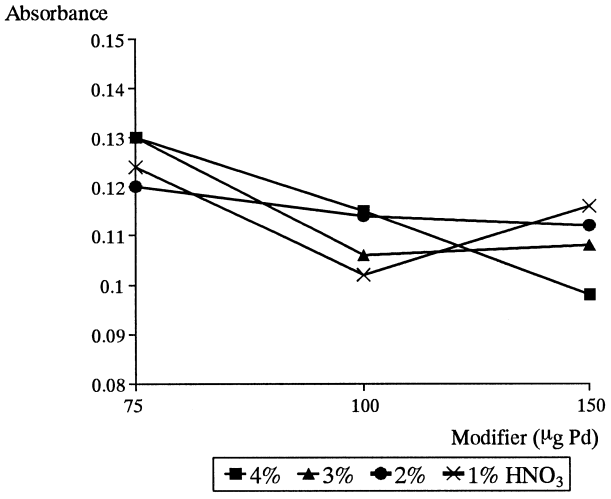

Fig. 1. Effect of different amounts of matrix modifier and nitric concentration on the atomic absorption signal.

range from 20 to 300 ng ml^{-1} in milk and from 4 to 60 ng ml^{-1} in the assay. The adjusted linear equation and correlation coefficient obtained were: $y = 0.00458 + 0.00279x$; $r = 0.997$; where y = absorbance and x = lead concentration in assay (ng ml^{-1}).

3.2.2. Detection limit (ACS, 1983)

The detection limit, defined as the lead concentration corresponding to three times the standard deviation of eight reagent blanks, was 1.0 ng ml^{-1} in the assay, which corresponds to 5.0 ng ml^{-1} in milk.

3.2.3. Precision

The instrumental precision, estimated from five consecutive measures of the same milk sample ($x = 11$ ng ml^{-1}), was 8.7%. It should be pointed out that the injection of the sample was manual. The precision of the method was estimated from an analysis of 10 homogeneous aliquots of milk. The intra-assay precision (aliquots of a sample measured during the same assay session) and inter-assay precision (aliquots of the same sample measured in different days) were also estimated. The values obtained were: intra-assay 11.7% and inter-assay 14.9%

3.2.4. Accuracy

In order to check the accuracy of the proposed method, recovery assays were undertaken to assess the accuracy of these measurements since no appropriate reference materials were available. To do this, milk sample aliquots whose lead contents had been already determined were spiked with known amounts of this analyte (19 ng ml^{-1}). The recovery percentage obtained was $109.8 \pm 5.4\%$.

4. Conclusions

From the values of the analytical parameters of the proposed method it can be concluded that it is useful for determining lead in human milk. The main advantages of the direct determination are a reduced risk of losses and also of contamination.

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