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Evaluation and Determination of Heavy Metals (Mercury, Lead and Cadmium) in Human Breast Milk

Atousa Abdollahi[1], Fariba Tadayon[2] and Mooud Amirkavei[3]

[1] Department of Environmental Chemistry, Islamic Azad University Khorasgan (Isfahan) Branch, 8159-158 Isfahan, IRAN
[2] Department of Chemistry, Islamic Azad University North Tehran Branch,Tehran, IRAN
[1] Department of Environmental Chemistry, Islamic Azad University Khorasgan (Isfahan) Branch, 8159-158 Isfahan, IRAN

Abstract. Mercury, Lead and Cadmium were determined in 100 samples of human breast milk samples from urban and rural mothers in Isfahan (IRAN). A questionnaire about area of residence, nutrition, smoking habits, and dental fillings was filled out by the lactating mothers. The combination of nitric acid, hydrogen peroxide and perchloric acid was found to be one of the most suitable acids in wet digestion of milk. Cold vapor atomic absorption was used to determine the mercury content in milk after wet digestion. The effect of concentration of nitric acid, influence of flow rate and tin(Π) chloride were investigated. The mean concentration of mercury in human breast milk samples was 0.96 ppb. Extraction of Pb and Cd were performed with ammonium pyrrolidine dithiocarbamate (APDC) to methyl isobutyl ketone (MIBK) and were determined by Flame Atomic Absorption Spectrometry. The factors influencing, the complex formation, pH, time and buffer were optimized. The mean concentration of Pb and Cd in human breast milk was 0.0147 and 0.0121 ppm, respectively. The maximum concentrations were found in breast milk of rural mothers.

Keywords: Heavy metals, Mercury, Lead, Cadmium, human breast milk, Cold vapor atomic absorption, Flame Atomic Absorption Spectrometry

Introduction

Human milk is the main source of food for infants during the first four to five months of their lives. Breast milk can, however, also be a pathway of maternal excretion of toxic elements. Breast milk consists of some hazards and may include heavy metals and other contaminates, which could have harmful effects on nervous system (Massaro, 1997; Jarup, 2003; Wong and Lye, 2008). Lead and Mercury are toxic to the developing brain, lead has subtle effects on neurological functions, and including learning, memory, and attention span (Hayano et al., 1996). Pb is one of the neurotoxicants and its concentration in breast milk is mostly higher than dose of Hg. Pb that was accumulated in the mother's bones in the past, is released along with calcium into her blood, and frequently pollutes her breast milk (Ettinger et al., 2006). It has been said that, 36–80% of all blood Pb in breast-fed infants comes from mother's milk during the first three months of their lives (Gulson et al., 2003). Harmful effects of mercury, include brain damage, mental retardation, seizures and inability to speak. Mothers most likely to be exposed to metallic mercury from mercury released from dental fillings. Methyl mercury can build up in certain fish to much higher levels and these fish may then be eaten by mothers (Oskarsson et al., 1996). Cadmium is toxic to the male reproductive system, the kidneys, and the brain (Hayano et al., 1996). Cd is a famous human carcinogen. It can also influence brain growth in infants, and it has been estimated that it might increase the risk of premature delivery (Nishijo et al., 2002; Jarup, 2003; Schoeters et al., 2006). Unlike Hg and Pb, Cd is very little transferred through the placenta and only a small percentage reaches breast milk (Vather et al., 2001). Researchers found a strong correlation between the amount of toxic metals in the mother's milk samples and amount of these elements in environment and food.

There are two reasons for learning about the toxic metal levels in the breast milk: first, as a pathway of exposure, and second, as an indicator of likely prenatal exposure (Jarup, 2003).

The aims of this study were to measure Cd, Pb and Hg concentrations in the breast milk of healthy lactating women who were living in Isfahan, a big city in Iran and rural areas and to investigate the effect of mother's age, diet, lifestyle, smoking habits in the family on the concentration of these heavy metals.

Materials and Methods

All reagents used were of analytical reagent grades and were purchased from the Merck Company (Darmstadt, Germany). Mercury Analyzer, model MAS-50A Perkin Elmer was used for mercury measurment.

A Perkin Elmer atomic absorption spectrometer, model 2380 was used for other metal measurements throughout this study. Perkin Elmer hollow cathode lamps for Pb, Cd and Cr were used as light sources which operated, respectively, at current of 10, 6 and 25mA, wavelength of 217, 228.8 and 357.8nm with a spectra bandwidth of 0.7, 0.7 and 0.7nm, as recommended by manufacturers. The flame was optimized. The pH measurements were carried out with a Metrohm pH meter (model 632, Switzerland).

Results and Discussion

Different parameters affecting the complex formation, extraction and determination were optimized by using the uni variable approach. Four different methods of wet digestion were used to determine the matals in milk samples. The results obtained are summarized in Table 1. The effect of dilution on the recovery of Pb and Cd was studied by varying the volume in the range of 10-100 ml, and keeping the other variable constant. The results demonstrated in Table 2. The effect of pH on the extraction of 0.05 ppm of Pb and Cd was studied. The results demonstrated in Fig. 1.

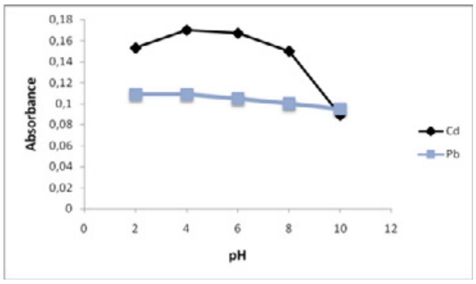

Fig. 1. The effect of pH on the extraction of 0.05 ppm of Pb and Cd

The effect of ammonium pyrrolidine dithiocarbamate (APDC) on the extraction of Pb and Cd was also investigated. Some experiments were performed by using different volumes of APDC (2%). The volume of 4 mL APDC 2% was used. The effect of the extraction time was examined in the range of 0-5min with constant experimental conditions. Two methods of preparation were applied to determine the mercury (Table3).

The effect of flowrate and amount of $SnCl_2$ was studied for Hg (Fig.2 and Fig.3).

Table 1. Comparison of 4 methods for wet digestion

	HNO_3+H_2O_2+$HClO_4$	HNO_3+H2SO4	HNO_3	CCl_3COOH
Hg	95.82±1.14	92.50±0.50	87.23±0.92	50.20±1.50
Pb	88.73±0.63	80.99±1.13	82.45±0.69	88.11±0.89
Cd	78.09±0.79	74.15±0.94	70.34±1.34	67.15±1.47

Table 2. Effect of dilution on recovery (n=3, recovery = mean ± SD)

	10ml	25ml	50 ml	100 ml
Pb	54.78±1.76	88.73±1.13	90.45±2.34	95.20±0.49
Cd	62.25±1.17	78.15±1.94	81.14±1.34	92.55±0.47

Table 3. Comparison of 2 methods for Mercury

Mercury added (μg Hg)	Method 1 recovery mean ± SD	Method 2 recovery mean ± SD
0.05	87.99±5.28	96.75±5.93
0.1	84.03±2.43	95.08±8.51
0.2	88.84±7.26	98.40±2.77
0.5	88.82±1.14	98.00±1.73
1	86.35±2.06	97.66±2.03

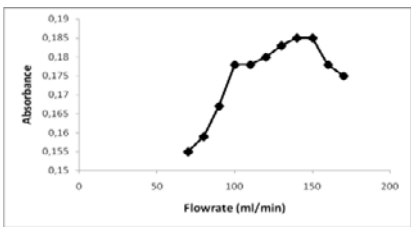

Fig. 2. The effect of flowrate on the absorbance signal of Hg.

The effect of other ions on the preconcentration and determination of analytes was examined.

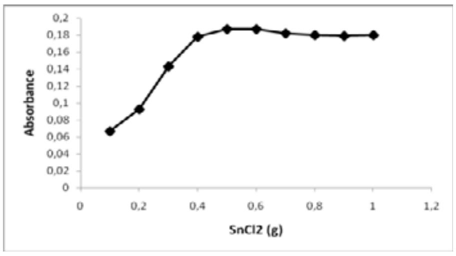

Fig. 3. The effect of $SnCl_2$ on the absorbance signal of Hg.

The concentration of metals in urban and rural mothers samples was investigated and the maximum concentrations were found in urban mothers samples. Fig.4 summarized the results and indicates the concentration of metals in the human breast milk samples of Isfahan.

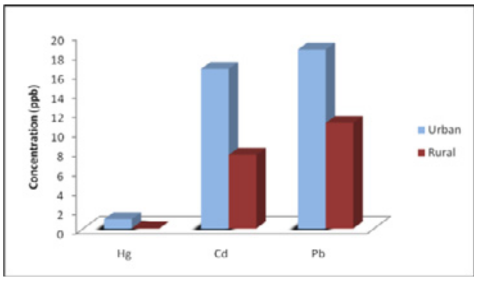

Fig. 4: The concentration of metals in breast milk of urban and rural mothers

Conclusion

The results show a correlation between the amount of toxic metals in the mother's milk samples and amount of these elements in environment and food. Smoking and dietary habits were the main factors related to heavy metal levels in breast milk. Our results entail the need to survey these pollutants in milk. It can be concluded that this method can be used for inexpensive and simple evaluation of metals contaminants in milk samples.

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