Simultaneous measurement of lead and cadmium in the milk distributed in Tehran's schools by differential-pulse anodic stripping voltammetry

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ABSTRACT:

Milk is one of the most important foods in the dietary regimen of children which can be contaminated with toxic elements such as lead (Pb) and cadmium (Cd). In this study, the concentrations of Cd and Pb were determined in the milk distributed among school-aged children in Tehran. Differential-pulse anodic stripping voltammetry (DPASV) method using hanging mercury drop electrode (HMDE) was performed. The obtained limit of detection (LOD) and limit of quantification (LOQ) were 0.31 ppb and 1.03 ppb for Pb and 0.09 ppb and 0.32 ppb for Cd, respectively. The concentration of Pb and Cd in the examined milk was 3.34 ± 0.63, and 0.42 ± 0.47 ppb, with recovery range between 85-109% and 91-112%, respectively. The results demonstrated that the levels of Pb and Cd were considerably less than the maximum residue limit (MRL), mentioned by the regulatory organizations.

Keywords: differential-pulse anodic stripping voltammetry (DPASV); milk; cadmium; lead.

1. Introduction

Malnutrition in school-aged children is a health-related critical issue which can affect learning ability and performance. Due to nutritional and immunological characteristics, milk is considered as a complete food in human regimen [1], which provides both macronutrients (lipids, carbohydrates, proteins) and micronutrients (vitamins, inorganic elements such as calcium and enzymes) for human especially in childhood. It is the only supplying source of food in infants and the most important component in growing children's dietary regimen [2]. School milk program is one of the well-known strategies to reduce the risk of malnutrition in school-aged children. In spite of these benefits, milk can be considered as a well-known and abundant source of toxic elements such as lead (Pb) and cadmium (Cd). These toxic elements can be transferred in milk through animal-polluted nourishment sources, manufacturing process, and/or packaging materials [1, 3]. The absorption of these toxic elements from foods is age-dependent, and children show the highest absorption amounts [3, 4].

It was reported that Pb is accumulated in the body through the lifetime and may result in serious health effects on the growing children, including concentration problems, mental retardation (e.g., reading and learning disabilities), hypertension, kidney damage, and hearing degradation [5]. Similar to Pb, cadmium can affect the brain development [6] and induce kidney damage [7], as well. Cd and Pb are classified as category 1 and 2A carcinogens, respectively by the International Agency for Research on Cancer (IARC) [4].

In recent years, due to toxic effects of lead and cadmium and the importance of milk in dietary regimens of children, many highly-sensitive analytical methods have been developed to detect and quantify heavy metals in milk and other dairy products. Inductively coupled plasma–atomic emission spectrometry (ICP–AES), inductively coupled plasma–mass spectrometry (ICP–MS), dynamic reaction cell combined with ICP-MS (DRC-ICP-MS), electrothermal atomic absorption spectrometry (ETAAS), flame atomic absorption spectrometry (FAAS), X-ray fluorescence (XRF), anodic stripping voltammetry (ASV) and differential pulse polarography (DPP) are the analytical methods which are used to determine Cd and Pb in milks, water, dairy products and biological samples [1, 3, 8, 9]. Among analytical methods, differential-pulse anodic stripping voltammetry (DPASV) attracted more attention due to lesser cost of analysis, simpler application, and suitable detection limits at trace to ultra-trace levels [10-13].

Since, Tehran is considered as an industrial city with air pollution issues, the animal husbandries in this region can encounter with high level of lead and cadmium in their products. Thus, in the present study, we decided to monitor the level of Pb and Cd by DPASV in the distributed milk in Tehran's schools (SHAMA-milk) according to School Milk Program in Iran and compared to the obtained results with the reported amount of these elements in milk from other countries.

2. Materials & Methods

A packed pasteurized cow milk containing 2.5% fat called "SHAMA" with batch number of 10973-1580 was acquired from local supplier. Deionized water was obtained from OES® water purification system (Overseas Equipment and Services Inc., USA). Pb and Cd standard solutions (1 g/L) in nitric acid 0.5 M (Certipur), nitric acid 65% (Suprapur), potassium chloride 99.999% (Suprapur), acetic acid glacial 100% (Suprapur), ammonia solution 25%, and all other reagents (analytical grade) were purchased from Merck (Darmstadt, Germany).

The working standard solution was the 0.5 mg/L mixture of Cd and Pb in nitric acid 0.1 M and was prepared from Cd and Pb stock standard solutions (1 g/L) in nitric acid 0.5 M.

The quality control (QC) and analytical samples were prepared from working standard solution either in nitric acid 0.1 M or milk sample.

The concentration of QC samples was 5, 25, and 50 ppb. The ammonium acetate with pH = 4.6 ± 0.2 was used as buffer solution which was made by the addition of 55.5

mL glacial acetic acid and 37 mL ammonia to 500 mL volumetric flask by adjusting the volume to 500 mL by deionized water.

The Metrohm voltammetry trace analysis system consisted of 746 VA Trace Analyzer with 747 VA Stand and also 685 Dosimats (Zofingen, Switzerland) was used. The analyzer was equipped with hanging mercury drop electrode (HMDE) as working electrode, saturated calomel electrode (SCE) as the reference electrode and platinum (Pt) electrode as auxiliary electrode. The data acquisition and analysis were performed by the software of the instrument.

The Pb and Cd were determined simultaneously via DPASV. The mixture of prepared sample, deionized water, and buffer solution (5:5:1 v/v) was added to measuring vessel of a 747 VA Stand and the analytes were determined according to the application bulletin 231/2e method of apparatus database. The standard addition technique was performed by adding two different volumes of working standard solution. The instrumental setting and analysis conditions for determination of Pb and Cd are shown in table 1. All experiments were done at room temperature.

Several parameters were considered to validate the analytical procedure including sensitivity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), linearity and recovery.

The recovery of QC samples was determined for 3 replicates. Statistical evaluation of obtained recovery was performed through the expanded uncertainty.

The precision was obtained through the determination of relative standard deviation (RSD) of the QC samples (3 replicates) over one day. HORRAT parameter was used for the analysis of obtained RSDs. This factor is defined by dividing the obtained RSD to calculated RSD according to Horwitz equation. The acceptable range for repeatability is 0.3 - 1.3 [14].

Table 1. The analysis conditions of determination method.	
Drop size	4
Vadded volume	6 mL
Stirrer/RDE	2000 rpm
Purge time	300 s
Pulse amplitude	0.05 V
Deposition potential	-1.15 V
Deposition time	90 s
Equilibration time	10 s
Start potential	-1.15 V
End potential	0.05 V
Voltage step	0.006 V
Voltage step time	0.1 s
Sweep rate	0.06 V/s
Peak potential (Cd)	-0.56 V
Peak potential (Pb)	-0.38 V

The difference between true value and measured concentration of three replicates of QC samples was used to determine accuracy.

The LOD and LOQ of each analyte were determined as 3 and 10 times of SDb/R expression, respectively. The SDb is the standard deviation of 5 replicate measurements of blanks and R is the mean of recovery [15].

Sensitivity of each analyte is calculated by multiplying the slope of its calibration curve by the related recovery.

In order to estimate the sample size, the PASS (Power Analysis and Sample Size) software (PASS 2008. NCSS LLC., USA) was used. Pooled SD was given to PASS software, and the sample size was calculated by using confidence intervals with tolerance probability method.

All the glass apparatus were soaked in nitric acid 0.1 M for 16 - 24 h and rinsed with deionized water to reduce the risk of contamination [2]. A volume of 50 mL of milk samples were dried using oven (Precision Scientific, USA, Model: 25EM) at 50 - 60 °C for 4 days. Dried samples were ashed at 500 °C with furnace (SYBRON/KERR, USA, Model: 666) for 16 h. If the color of the obtained ash was not white, it would be mixed with 25 mL of deionized water and 0.5 mL of nitric acid (65%). The mixture was ashed at 500 °C for 1 - 2 h with furnace again. This process was repeated until the white ash was obtained. The white ash residue was dissolved in 5 mL nitric acid 1 M by means of heat and diluted with 0.1 M nitric acid solution [16]. The prepared samples were analyzed in duplicate, and the data were presented as mean ± standard deviation.

3. Results

The validation parameters are presented in table 2.

The obtained recovery for Pb and Cd was between 85 - 109%, and 91 - 112%, respectively. According to the expanded uncertainty, the recoveries showed no significant differences with 100% recovery. Since the acceptable range for recovery was mentioned between 70 - 125% when the concentration of analyte was greater than or equal to 10 ppb or less than 1000 ppb [17], the recovery of the method was acceptable for the determination of Pb and Cd.

The precision of the method was another parameter which showed the acceptable range according to HORRAT parameter ($0.3 \leq$ HORRAT ≤ 1.3).

The obtained LOD and LOQ by this method were 0.31 ppb and 1.03 ppb for Pb as well as 0.09 ppb and 0.32 ppb for Cd, respectively. The obtained detection limits in this study by DPASV method were considerably lower than the previously reported atomic absorption spectrometry (AAS) techniques and were comparable with DRC-ICP-MS (Table 3).

Table 2. The validation parameters for the determination of lead (Pb) and cadmium (Cd).

Parameters	Cadmium	Lead
LOD (ppb)	0.0949	0.3094
LOQ (ppb)	0.316	1.0313
Recovery (%)	91.16- 112.51	85.39- 109.34
Interday Precision (RSD %)	7.96	7.26
Intraday Precision (RSD %)	5.38	4.71
Sensitivity (nA/ppb)	2.94	1.54

The calibration curves showed the reasonable linearity (r > 0.99) in the selected range for both Cd and Pb.

The calculated sensitivity for Cd and Pb was 2.94 nA/ppb and 1.54 nA/ppb, respectively. The sensitivity of this method for Cd was two times higher than Pb.

In relation to Commission Regulation, these method validation parameters were in acceptable range for the analysis of Pb and Cd in foodstuffs [18].

According to the results of PASS software the sample size of eight can determine Cd and Pb in milk with 95% confidence interval and 99% tolerance probability.

The concentration of Pb and Cd was determined in the "SHAMA" milk which was distributed in Tehran's schools in Iran was 3.34 ± 0.63 and 0.42 ± 0.47 ppb, respectively (Fig. 1).
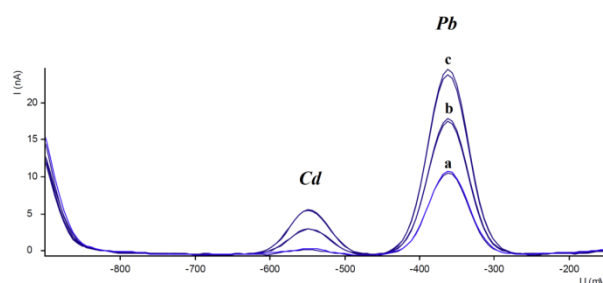

Figure 1. Determination of cadmium (Cd) and lead (Pb) simultaneously by Differential-Pulse Anodic Stripping Voltammetry (DPASV) in milk sample. Sample curve (a), first standard addition curve (b) and second standard addition curve (c). Deposition potential = -1.15 V, deposition time = 90 s, pulse amplitude = 0.05 V.

The FAO/WHO expert committee reported the acceptance range of Cd as 10 - 50 ppb in most foods. So, in this study, the 10 ppb is considered as maximum residue limit (MRL) for Cd [21].

In case of Pb, according to the CE Regulation the MRLs in raw and fat milk were 20 ppb and 100 ppb, respectively [22]. The mean concentrations of Pb and Cd in "SHAMA" milk were much lower than the established and considered MRL for Pb and Cd, respectively.

Table 3. Comparisons of LOD values of different published methods.

LOD (ppb)		Method[a]	Sample	References
Cadmium	Lead			
0.5	3.40	AAS	Breast milk	[6]
0.3	1.6	AAS	Infant cereal	[4]
0.63	7	ICP-AES	Milk products	[19]
0.08	0.5	DRC-ICP-MS	Bovin milk	[1]
0.30	1.7	PSA	Milk and fermented milk products	[20]
0.09	0.31	DPSAV	Pasteurized milk	This study

a. AAS: Atomic Absorption Spectrometry, ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometry, DRC-ICP-MS: Dynamic Reaction Cell Combined with ICP-MS, PSA: Potentiometric Stripping Analysis, DPSAV: Differential-Pulse Anodic Stripping Voltammetry.

The provisional tolerable weekly intakes (PTWIs) of Pb and Cd, for all groups, are 0.025 and 0.007 mg/kg, respectively, according to the World Health Organization (WHO) [9]. There are wide variations in the reported range of Cd and Pb in the literature. Some of these data are summarized in table 4. The concentrations of Cd and Pb in "SHAMA" milk are comparable with the reported level of these heavy metals in different countries.

4.Discussion and Conclusion

Since milk is a major source of nutrition in childhood, it is important to monitor the level of trace elements in it. The trace elements such as Pb and Cd can produce serious health effects on the kidney and nervous system of growing children. Pb and Cd are classified as category 2A and 1 carcinogens, respectively, by the International Agency for Research on Cancer (IARC) [4].

Table 4. The reported concentrations (ppb) for lead (Pb) and cadmium (Cd) in milk and milk products in different countries.

Country	Samples	Concentration (ppb)		Reference
		Cd	Pb	
Romania	Powder milk	0.001	0.16	[19]
	Fresh cow milk	0.004	0.12	
	Pasteurized milk	0.003 - 0.005	0.04 - 0.11	
Spain	Human milk	1.15 - 1.48	12.97 - 18.72	[6]
Canada	Milk-based formulae	0.03 - 1.26	0.14 - 2.46	[23]
India	Goat milk	0.084 – 0.092	0.021 – 0.031	[24]
	Cow milk	0.088 – 0.092	0.016 – 0.020	
	Ewe milk	0.064 – 0.080	0.014 – 0.020	
	Buffalo milk	0.042 – 0.052	0.010 – 0.020	
	Human milk	0.024 – 0.044	0.006 – 0.012	
South Korea	Plain milk	2.357 - 2.403	3.274 - 3.426	[25]
	Skimmed milk	2.196 - 2.244	13.822 - 13.898	
Turkey	Raw milk	0.180 - 0.398	5.32 - 9.94	[26]
Iran	Cow milk	0.28 - 3.43	1.84 - 20.70	[5]
	Goat milk	0.64 - 5.40	2.91 - 14.40	
	Sheep milk	0.79 - 6.11	4.61 - 30.50	
	Buffalo milk	0.18 - 1.34	3.12 - 13.60	
	Raw milk	---------	1 - 46	[27]
	Pasteurized milk	0.42	3.34	This study

In this study the concentration of Cd and Pb in "SHAMA" milk was monitored by simple and rapid and validated DPASV method according to Commission Regulation (EC) No 333/2007. This milk is distributed in Schools of Tehran according to the school milk program.

The results demonstrated that the concentration of Pb and Cd were less than the established Pb MRL (20 ppb) and the considered Cd MRL (10 ppb). Although this milk product is not the only source of milk and dietary regime in children, it is important to monitor the concentration of Cd and Pb in other brands and milk products to prevent the accumulation of these elements and their undesirable effects.

Acknowledgment

Conflict of interest

None.

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