Original Article

Simultaneous Determination of Lead, Cadmium, Copper and Zinc in Infant Formula by Anodic Stripping Voltammetry

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Abstract

Determination of trace elements in food is of great importance, as some of them have nutritional significance, whilst others are toxic. Long-term exposure to low levels of toxic metals could be important, especially in relation to children.

Differential Pulse Anodic Stripping Voltammetry (DPASV) is applied to simultaneous determination of lead, cadmium, copper and zinc in dry ash infant formula in nitric acid at $450\pm50°C$.

Determination of the elements was made in acetate buffer (pH 4.7) with a scan rate of 60 mVs^{-1} and a pulse height of 50 mV by Hanging Mercury Dropping Electrode (HMDE). The solution was stirred during pre-electrolysis at -1150 mV (vs. Ag/AgCl) for 90 s and the potential was scanned form -1150 mV to +100 mV(vs. Ag/AgCl). Precision of the method, expressed by relative standard deviation for lead, cadmium, copper and zinc, was calculated as 4.5%, 3.5%, 3.2% and 5.6%, respectively. Under these conditions, the limits of detection were 0.005, 0.005, 0.01 and 0.05 mg/kg for lead, cadmium, copper and zinc, respectively. Concentrations of lead, cadmium, copper and zinc in one of the infant formulas available in Iran were 0.384 ± 0.222, 0.359 ± 0.215, 4.436 ± 0.811 and 37.426 ± 8.951 mg/kg (mean±SD), respectively.

In our study the concentrations of lead and copper were found significantly higher than those of standard and labelled values (P<0.001), respectively. Therefore, infant formula manufacturers, by virtue of the role of their products in the infants' nutrition, have been acutely aware for sometime of the need to guard against any heterogeniety and contaminants.

Keywords: Infant formula; Trace element; Lead; Cadmium; Copper; Zinc; Voltammetry; Anodic stripping.

Introduction

Determination of trace elements in food is of great importance, as some of them have

nutritional significance, whilst others are toxic. Zinc and copper are the essential trace elements which their deficiency cause impairment of functions and excess amounts will be toxic. Cadmium and lead are heavy metals which are widely distributed, naturally occurring, and potentially toxic elements. With increasing

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industrial use, environmental pollution and associated toxic exposure, concern has increased about their long term exposure and potentially toxic effects on human health specially infants and young children which are at the pick of growth (1, 2).

Frequently used methods for determination are atomic absorption spectrometry, neutron activation and inductively coupled plasma atomic emission spectroscopy. The specialized and expensive technique of neutron activation analysis has been successfully applied for this purpose too. Other accessible techniques capable of multielement determination such as ICP-AES and X- ray fluorescence are very expensive and often do not offer sufficient sensitivity usually required in food, biological and environmental samples (3).

Differential Pulse Stripping Voltammetry (DPSV) is relatively inexpensive and is one of the most sensitive and selective techniques in the determination of trace amounts of metals at natural levels (4-10). In this study Cd, Pb, Cu and Zn are determined in one of the commercial infant formula available in Iran, by the DPASV technique. Up to our knowledge, there is no report on the trace elemental content of the infant formulas in the country so far.

Experimental

Apparatus

The polarograph was Trace Analyzer model 746 instrument (Metrohm AG Ltd., Switzerland). The cell was a three-electrode system with an Ag/AgCl electrode as reference electrode, a hanging mercury drop electrode (HMDE) as the working electrode and a platinum electrode as auxiliary electrode.

Reagents and solutions

The tartaric acid, CH_3COOH, CH_3COONa, $Pb(NO_3)_2$, $Cd(NO_3)$, $Cu(NO_3)_2$, $Zn(NO_3)_2$ and HNO_3 used were from Merck (pro- analysis grade).

The metal stock solutions (1 g/L) were prepared in 0.005 M HNO_3. The acetate buffer was 0.2 M and pH 4.7 containing 0.2 M tartaric acid, which served as a supporting electrolyte.

Glassware

All glassware were soaked in 2 M nitric acid for at least 7 days, washed three times with distilled deionized water, soaked in 0.1 M hydrochloric acid until ready for use. in distilled deionized water and finally soaked

Sample preparation

A sample weighed 2 g among with 5 mL nitric acid 65% were transferred into a cleaned glass vessel. The sample was evaporated to dryness on a hot plate. Temperature of the hot plate was gradually increased form low to maximum over a period of 1-2 h. The charred sample was transferred to a muffle furnace and ashed at 450-500°C for 24 h. The residue was wet with 1 mL nitric acid 65% and 9 mL distilled water, and then the crucible was heated on a hot plate near boiling to complete dissolution of the residue. The sample was then quantitatively transferred to a 25 mL volumetric flask and diluted to volume.

Voltammetric determination

A portion of 5 mL acetate buffer and 0.5 mL of the prepared sample were transferred into polarographic cell, deoxygenated for 120 s with high–purified nitrogen. A fresh mercury drop as working electrode (HMDE) was extruded, then the stirrer and electrolyze were started for 90 s at –1150 mV vs Ag/AgCl. The stripping voltagram for zinc, cadmium, lead and copper was recorded after a rest period of 10 s after stirrer stopping. The potential was scanned from –1150 mV to +100 mV (vs. Ag/AgCl) with a scan rate of 60 mVs^{-1} and a pulse height of 50 mV. The stripping voltammetric measurement was repeated (each measurement must be done at least twice), 100 µL of standard metal solutions was added (standard addition calibration method), and the new voltagram was recorded again. Voltagram of these elements is shown in Figure 1. The concentrations were calculated after the second standard addition, using linear regression method.

Statistical analysis

Statistical analysis was performed using SPSS software version 12. The data are expressed

Table 1. Determination of lead, cadmium, copper and zinc in infant formula (n = 160).

	Lead (mg/kg) Mean ± SD	Cadmium (mg/kg) Mean ± SD	Copper (mg/kg) Mean ± SD	Zinc (mg/kg) Mean±SD
Infant formula	0.384 ± 0.222*	0.359 ± 0.215	4.436 ± 0.811*	37.426 ± 8.951
Labelled values Or Standard value	0.2	-	3.0	38.0

* P < 0.001   One sample t-test

as the mean±SD. The concentrations were calculated by the method of linear regression method. For the comparison of concentrations with the allowable value of standard or labels, one sample *t* test was used. A *P* value less than 0.001 were considered as significant.

Results

Table 1 shows pb, cd, cu and zn content of the sample studied.

Detection limits for lead, cadmium, copper and zinc were 0.005 mg/kg, 0.005 mg/kg, 0.01 mg/kg and 0.05 mg/kg, respectively. Precision of the method expressed by relative standard deviation were 4.5%, 3.5%, 3.2% and 5.6%, respectively.

Discussion

Sample preparation
The problem of mineralisation of organic matrices in samples has been discussed (9). Digestion by UV irradiation is suitable for samples with low organic matter but is not suitable for those with high organic matter as infant formula, so wet digestion or dry ashing can be used (9). Basically, wet digestion and dry ashing are the most common techniques, which have advantages and disadvantages. Wet digestion is advantageous because of its applicability to a wide variety of samples, with few volatilization problems, but it requires relatively large volumes of potentially hazardous reagents, with consequent high background values and safety precautions. On the other hand, dry ashing can be run without constant attention, thus leading to processing of large number of samples, and it does not require large volumes of reagents.

However, losses by volatilisation can occur with this procedure but according to Gorsuch, as long as the ashing temperature does not exceed 500°C, volatilisation losses should not occur for most elements. Dry ashing is simpler to carry out and results are less contamination of the sample from the reagents (11, 12).

Determination of the elements
The reliable determination of zinc, copper, cadmium and lead by ASV is dependent on the pH of the solution. According to previous studies optimum pH is 4.7 (13, 14) and therefore determination of the elements was done in pH 4.7.

In this study, an interference was observed which could produce false positive results in lead or cadmium determinations by the ASV method. It is known that tin yields a peak at a potential between those of lead and cadmium (15, 16). We eliminated this interference by dry ashing with nitric acid, which oxidizes Sn (II) to Sn (IV).

Metastannic acid ($Sn(OH)_2O$) or SnO_2 formed is insoluble in nitric acid (17). Also tartaric acid in the supporting electrolyte is intended to complex Sn (IV) and renders it electroinactive (15).

Other interference was Cu-Zn intermetallic compound, which can cause error in the analysis of Cu or Zn by ASV. The effects of this intermetallic compound on the practical application of ASV in the analysis of Cu and Zn have been generally disregarded (18, 19). This interference may be eliminated by adding Ga which appears to form intermetallic compounds itself, that does not cause interference (19). Other method in solving this problem is to use Neural Networks (20). In present study, the errors due

to interferences were minimized by the standard addition method.

We found in this study that the concentrations of lead and copper were significantly more than standard and labelled value (P<0.001), respectively (Table 1). Thus, the results indicated that more vigorous measures should be take on the quality control of the similar products in the Iranian market.

Acknowledgments

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