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Original Article

ANALYTICAL METHOD VALIDATION OF ICP-AES FOR ANALYSIS OF CADMIUM, CHROMIUM, CUPRUM, MANGAN AND NICKEL IN MILK

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ABSTRACT

Objective: The aim of this research was to validate inductively coupled plasma-atomic emission spectroscopy (ICP-AES) for quantitative analysis of cadmium (Cd), chromium (Cr), cuprum (Cu), mangan (Mn) and nickel (Ni) in milk products.

Methods: The heavy metals in milk were determined using ICP-AES at optimized wavelength. The method was validated by assessing several validation parameters which included linearity and range, accuracy, precision and sensitivity expressed by the limit of detection and limit of quantification. The validated method was then used for the analysis of milks commercially available.

Results: ICP-AES for determination of Cd, Cr, Cu, Mn, and Ni was linear over a certain concentration range with a coefficient correlation value of>0.997. The limit of quantification values of Cd, Cr, Cu, Mn, and Ni were 0.0047; 0.0050; 0.0066; 0.0061; and 0.0169 µg/ml, respectively. The precision of analytical method exhibited relative standard deviation (RSD) values of 3.18%; 4.17%; 3.05%; 2.93%; and 4.47% during repeatability test and 5.28%; 5.06%; 3.67%; 3.67%; and 11.17% during intermediate precision of Cd, Cr, Cu, Mn, and Ni respectively. The recoveries of these metals assessed using standard addition method were 92.25; 90.88; 102.87; 94.50; and 86.85%, respectively.

Conclusion: ICP-AES offered a reliable and fast method for the determination of heavy metals in milk products. The developed method could be proposed as an official method for determination of heavy metals in milk products.

Keywords: Validation, ICP-AES, heavy metals, Wet digestion, Milk

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INTRODUCTION

Milk is one of the most important food products taking into account its protein contents, carbohydrates, fats, enzymes, vitamins and minerals, either macro-minerals like calcium and magnesium or micro-components such as Fe, Zn and C which are essential to promote the growth and maintenance of human life [1, 2]. Milk can carry numerous xenobiotic substances coming from the extrusion of mammary glands, which in turn constitute risk factors [3]. In addition, due to the growing environmental pollution caused by the increase of industrial, agricultural and urban emissions, milk and other dairy products contain different amounts of toxic contaminants including heavy metals [4]. The regulatory agencies have determined the maximum levels of heavy metals allowable to be present in the products.

The heavy metals like cadmium (Cd), chromium (Cr), cuprum (Cu), mangan (Mn) and nickel (Ni) present in high levels resulted some adverse effects on human health because of its cumulative effect. These heavy metals present in milk and other dairy products, even in the low levels, caused metabolic disorders which corresponded to some health problems like heart failure, weakness and cancer. Cd is reported as one of the toxic metals even at low concentrations and is classified as human carcinogen by International Agency for Research on Cancer [5]. The maximum tolerable daily intake of Cd in food products is 1.0–1.2 µg/g food. The toxicity of Cr depends on the oxidation state, while Cu is linked with certain cancer in animal models [6]. Chromium (III) is one of the essential minerals needed to maintain the function of normal physiological function, while Cr(VI) is toxic [7]. Mangan (Mn) has been reported to have cumulative properties. The chronic manganese excess can affect in central nervous system, having symptoms similar to Parkinson's disease [8]. The toxic effect of Ni(II) for human has been reported. Nickel could destroy the central nervous system, heart and kidneys, reduced the immunological capacity, eczema, and allergic [9, 10]. As a consequence, the determination of these metals are required to assure the safety of milk and other dairy products.

Milk is widely consumed in the human diet, especially in early childhood, therefore, the control of trace elements in milk via determination of these heavy metals is needed by some regulators. Numerous methods have been used for the determination of heavy metals, namely flame atomic absorption spectrometry [11], graphite furnace atomic absorption spectroscopy [12], inductively coupled plasma-mass spectrometry [12], inductively coupled plasma emission atomic spectrometry [3], inductively coupled plasma optical emission spectrometry [13], atomic fluorescence spectrometry [14], potentiometric stripping analysis [1], stripping potentiometry [15], differential pulse anodic stripping voltammetry technique [16], flow injection spectrometric methods [17], capillary zone electrophoresis [18], polarimetry analysis [19], and the commonly used one is atomic absorption spectrophotometry [20]. The objective of this study was to validate simple ICP-AES for simultaneous determination of cadmium (Cd), chromium (Cr), cuprum (Cu), mangan (Mn) and nickel (Ni) in milk samples.

MATERIALS AND METHODS

Materials

Milks from different brands were purchased from the supermarket around Universitas Gadjah Mada Yogyakarta, Indonesia. The standard solutions of cadmium (Cd), chromium (Cr), cuprum (Cu), Mangan (Mn), and nickel (Ni) with concentration of 1000 µg/ml as well as nitric acid 65% and perchloric acid 70-72% were purchased from E. Merck (Darmstat, Germany), aquabidestillata was obtained from LPPT Universitas Gadjah Mada, Yogyakarta. Reagents and solvents used were of pre-analytical grade reagents.

Preparation of working solution

The working solution was prepared by diluting stock solution (1000 µg/ml) using diluting solvent (HNO_3 1.5%) to obtain a series of solution of Cd, Cu, Cr, Mn and Ni with concentrations of 0.01-1.0 µg/ml. These solutions were subjected to ICP-AES measurement using the condition as follows, Spectrometer: High resolution echelle polychromator and large format programmable array (L-PAD); RF Generator: 40 MHz free running; Output power: 1.1 kW; Peristaltic

pump: 1.0 ml/min; Nebulizer: Pneumatic (glass concentric); Spray Chamber: Glass cyclonic.

Sample digestion

Sample digestion was performed using wet digestion method according to Noviana *et al.* [21], with slight modification. A-5.0 ml milk was introduced into digestion flask, added with 14 ml of a mixture of HNO_3 65% and $HClO_4$ 70-72% (1:1 v/v). The solution was heated at the hot plate until a clear solution appeared. After cooling, the solution was made until 25.0 ml in a volumetric flask with aquabidestillata.

Validation of the analytical method

ICP-AES method was validated according to Eurachem guide as in Noviana *et al.* [21]. Some validation parameters namely linearity, range, sensitivity expressed with a limit of detection and limit of quantification, precision (repeatability and intermediate precision), and accuracy was assessed to evaluate its suitability for the intended use of the used method. The validated method was then used for the analysis of milk samples.

RESULTS AND DISCUSSION

Analysis of metals of Cd, Cr, Cu, Mn, and Ni using ICP-AES involved sample destruction using HNO_3-$HClO_4$ as destructive agents. The temperature used during wet digestion was set initially at 130°C and then ramped at 200°C. The digestion was stopped if the solution appear clear. Decomposition of the sample is essentially ensured by a common wet digestion procedure, which is performed under the synergistic effects of elevated temperature; however, heavy metals are stable even in high temperature. ICP-AES method was validated according to Eurachem guideline by evaluating some parameters of linearity and range, sensitivity, precision and accuracy.

The linearity of the analytical method was evaluated by making a series of standard solutions of Cd, Cu, Cr, Mn and Ni with certain concentration and correlating between the intensities of light emitted during analysis (y-axis) along with its concentration (x-axis). Each element emitted energy in several wavelengths which are characteristics to the transition from low energy levels into higher energy levels. Analysis of elements using ICP-AES is typically performed using one wavelength which provides maximum sensitivity. Therefore, in this study, some wavelengths were optimized and the selected wavelength was based on its capability to provide the highest slope (maximum sensitivity) and lowest %-intercept. For example, Cd was measured at three wavelengths, namely 214.438 nm, 226.502 nm, and 228.820 nm. Fig. 1 revealed the relationship between Cd concentration (x-axis) and intensity of light emitted (y-axis), along with the coefficient of correlation (r). The wavelength of 226.502 nm was selected for further analysis of Cd, as the slope maximum (10483.889) with minimum intercept value (43.339). Table 1 listed the linear regression for the relationship between the concentrations of metals and intensities at optimized wavelength, along with r-value and concentration range. All r-values were higher than 0.99 which indicated that linear relationship existed between concentration and intensities at a certain concentration as in table 1[22]. In addition, %-intercept values were also low (<2%).

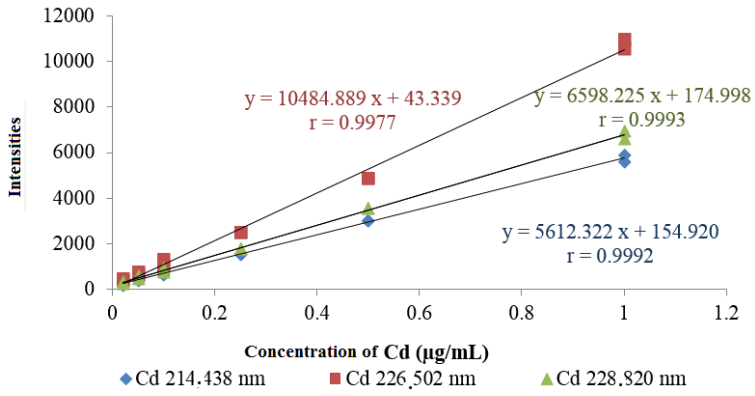

Fig. 1: The relationship between Cd concentration (x-axis) and intensity of light emitted (y-axis) at wavelengths of 214.438 nm, 226.502 nm, and 228.820 nm

Table 1: The linear regression for the correlation between the concentration of metals (x) and intensities (y) at optimized wavelength along with the coefficient of correlation (r)*

Metals	Wavelength	Concentration range (µg/ml)	Linear regression	r-value
Cd	226.502 nm	0.01-1.0	y = 10483.889x+43.339	0.9977
Cr	267.716 nm	0.01-2.0	y = 8413.406x-33.868	0.9994
Cu	324.754 nm	0.01-1.0	y = 21882.269x-211.435	0.9974
Mn	257.610 nm	0.01-0.5	y= 81355.162x+100.877	0.9998
Ni	221.647 nm	0.01-1.0	y = 3964.189x+69.398	0.9998

*obtained from three replicates (n = 3)

The sensitivity of the analytical method was expressed by the limit of detection (LoD) and limit of quantification. The developed method should be revealed LoD values lower than maximum limits of these metals in milk. The detection limits found were 0.0047 µg/ml, 0.0066 µg/ml, 0.0015 µg/ml, 0.0018 µg/ml and 0.0051 µg/ml for Cd, Cu, Cr, Mn and Ni, respectively. In addition, LoQ values were 3.33 x LoD values of corresponding metals. The LoD values were lower than the maximum limits of metals allowed in milk. For example, according to Indonesian standard, the maximum limits of Cd in UHT milk and powder milk were 0.2 µg/g and 20 µg/g, respectively, therefore in can be concluded that ICP-AES was sensitive enough for determination of these metals in milk products. The precision of ICP-AES was evaluated by repeatability and intermediate precision using different days of analysis. The relative standard deviation (RSD) values were used for precision evaluation. The method was precise if RSD values obtained were lower than RSD Horwitz. Table 2 compiled RSD values of metals analyzed using the developed method either during repeatability test or during intermediate precision. The maximum RSD horwitz for analyte level of 1.0 µg/ml was 16%. All RSD values during precision studies were lower than 16% [23], therefore, ICP-AES method was precise enough for quantitative analysis of Cd, Cu, Cr, Mn and Ni in milk samples.

Table 2: The relative standard deviation (RSD) values of Cd, Cr, Cu, Mn, and Ni in milk analyzed using ICP-AES (n =6)

Heavy Metals	RSD values (%)	
	Repeatability	Intermediate precision
Cd	2.19%	5.28%
Cr	3.71%	5.06%
Cu	3.05%	3.67%
Mn	2.93%	3.67%
Ni	5.01%	11.17%

The accuracy of the analytical method was evaluated by recovery percentage of analyte added into milk samples using the standard addition method. Three levels of concentrations were used for accuracy studies. Table 3 compiled recovery percentages on metals as analyzed using ICP-AES along with RSD values obtained during three replicates for each levels. Association of Official Analytical Chemists (AOAC) set up that the recovery percentage of analyte at level 1.0 ppm was in the range of 80-110% [21], therefore, the recovery percentages obtained using ICP-AES was acceptable, meaning that this method was accurate for determination of metals in milk. It was also stated that systematic errors do not significantly contribute for this method.

Table 3: The recovery percentages of Cd, Cr, Cu, Mn, and Ni in milk analyzed using ICP-AES (n = 3 for each concentration levels)

Metals	Recovery percentages*	RSD (%)
Cd	92.25±5.55	6.02
Cr	90.88±2.33	2.56
Cu	102.87±5.21	5.06
Mn	94.50±5.32	5.63
Ni	86.85±6.62	7.62

*expressed as mean±SD.

Based on validation parameters evaluated, it can be concluded that ICP-AES was valid for quantitative analysis of Cd, Cu, Cr, Mn and Ni in milk samples. The validated ICP-AES method was then used for analysis of these metals in milik products, and the results were compiled in table 4. All milk products evaluated meet the standard, i.e. below the maximum concentration allowed by Indonesia standard.

Table 4: The concentration of Cd, Cr, Cu, Mn, and Ni in milk samples as analyzed using ICP-AES**

Samples	Cd (mg/l)	Cr (mg/l)	Cu (mg/l)	Mn (mg/l)	Ni (mg/l)
IC	0.3374±0.0022	0.1933±0.0026	0.4877±0.0090	0.6682±0.0039	0.1396±0.0045
IV	0.2380±0.0018	0.1344±0.0037	0.1693±0.0028	0.0288±0.0037	nd*
FS	0.2495±0.0056	0.1484±0.0033	0.1857±0.0041	0.1063±0.0023	nd*
US	0.2637±0.0019	0.1310±0.0039	0.1674±0.0008	0.0324±0.0029	nd*
UV	0.3175±0.0012	0.1665±0.0078	0.2033±0.0171	0.0193±0.0038	0.0789±0.0098

*nd = not detected; ** the concentrations of heavy metals were expressed as mean±SD from three replicates (n = 3).

CONCLUSION

Inductively coupled plasma-atomic emission spectroscopy has been successfully validated and used for the analysis of metals (Cd, Cu, Cr, Mn, and Ni) in milk products. The validation parameters meet the requirement. The levels of these metals in milk products were lower than those required by Indonesian standard.

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AUTHORS CONTRIBUTIONS

DA and AR performed research activities and prepared manuscript. AR designed research, analyzed data, and made critical thinking on the manuscript.

CONFLICTS OF INTERESTS

All authors have none to declare

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