



## Research Article

## Development and validation of atomic absorption spectrometry for the determination of zink and mercury analyzer for determination of Mercury in cream cosmetics

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## ARTICLE INFO

Received 10/03/2015  
 Received in revised form  
 25/03/2015  
 Accepted 25/04/2015  
 Available online 01/5/2015

## ABSTRACT

An atomic absorption spectrometry for the quantification of zink (Zn) and mercury analyzer for the quantification of mercury (Hg) levels in cream cosmetic were developed and validated. The method was validated for linearity and range, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The standard curves were linear over the concentration of 0.1-1.2 µg/mL (Zn) and 0.05-2.0 µg/L (Hg) with coefficient of correlation ( $r$ ) > 0.99. The detection limits obtained were 2.8614 µg/g sample (Zn) and 0.4749 ng/g sample. The quantification limit obtained were 9.5281 µg/g sample (Zn) and 1.5827 ng/g sample. The relative standar deviation (RSD) values found to be 8.67 % for its intraday precision and 9.89 % for its interday precision (Zn) and 12.69 % for its intraday precision and 7.17 % for its interday precision (Hg). These RSD values are lower than those required by RSD Horwitz unction. The mean recovery percentage was 94.28 % (for Zn) and 78.65% (for Hg). These developed methods were succesfully used for determination of Zn and Hg in cream cosmetics products.

**Key words:** mercury, zink, atomic absorption spectroscopy, mercury analyzer, cream cosmetic

### 1. Introduction

Cream is one of the cosmetic products. They are used throughout skin protection, skin therapy, and cosmaceutical (Epstein, 2009). Cream is defined as thickly liquid or half solid emulsion, which is oil in water or water in oil emulsion type. Cream contains oil, water, and absorbtion phase. Other components of cream are emolient (5-25 %), trigliseride (0-5 %), mineral oil (5-70 %), silicon oil (0,1-15 %), humectan (0,5-15 %), and preservative. The most commonly cream formulated was oil in water type (Ansel, 1989). Cream can contain heavy metals for specific purposes. Interaction of cream components to heavy metal cause physical or chemical change of cream such as texture, smell, and cream stability (Budiman, 2008).

Heavy metals cause a long-term risk on environmental and human health. They can accumulate to toxic levels. The cosmetic application on skin cause heavy metals to expose consumers to toxic levels of potentially hazardous chemicals (Blanc *et al.*, 1999). Heavy metal such as zink (Zn) can penetrate to human body through inhalation and absorbtion on the skin (Plum *et al.*, 2010). Zn usually formulated as physical sunscreen on cream cosmetic. Zn toxicity may be manifested by a variety of syndromes and effects are including vomitting, convulsion, and diarrhea (Manahan, 1994 ; Darmono, 1995). Zink in cosmetics can penetrate through the skin space. The absorbtion of Zn is influenced by skin pH, zink consentration, and the the of comsumen's skin. In cream cosmetic, zink can be found as ZnO, ZnCl<sub>2</sub>, Zn(CH<sub>3</sub>COO)<sub>2</sub>, and ZnSO<sub>4</sub> (Plum *et al.*, 2010).

Mercury (Hg) is one of the most reported heavy metals found in cream cosmetics and can be found as organic compound such as methyl mercury or anorganic compound such mercury chloroyde ( $\text{HgCl}_2$ ) (Darmono, 1992). Hg in cream cosmetic is usually used to brighten the skin colour by melanin syntesis inhibition (Giunta et al., 1983). Hg can be bound to the side active of tyrosinase on melanin synthesis so the production of this human pigmen will be inhibited (Junquiera et al., 2002). Hg will accumulate and cause degenerative disease in human helath. Besides, Hg will be acumulated on tubular kidney (Barr et al., 1973; Berlin, 1979; Bourgeois et al., 1986; Marzulli and Brown, 1972). Hg in cosmetics could penetrate through the skin space.

Atomic absorption spectrophotometry (AAS) using flame and nonflame atomization is the method of choice for determination heavy metals contained in cosmetics products (Vogel, 2001). Some specific instruments are designed for specific purposes. Recently, mercury analyzer technique is the method of choice for mercury determination contained in cosmetics. Its determination pinciple that is free state atom of mercury can be found at room temperature (Beaty and Kerber, 1993). Method validation is an important aspect in quantitative analysis. According to ISO/IEC 17025 (2005), method validation intends to guarantee that the method meets the acceptable criteria. The purpose of this paper is to validate AAS for the determination of zink nd mercury analyzer for analysis of Hg in cream cosmetics distributed in cosmetic shop in Yogyakartaarta.

## 2. Materials and Methods

### 2.1 Materials

Cream cosmetics were obtained from cosmetic shop in Yogyakartaarta. Zink and mercury standard solutions were supplied by Merck (Darmstadt, Germany). The other reagents were supplied by Merck (Darmstadt, Germany). All reagents were analytical grade. The water used as solvent was distilled and deionized. All glassware used were cleaned by soaking in detergent solution, then rinsing with disitilled water.

### 2.2 Digestion procedure

An approximately of 1 gram of cream cosmetic weighed into 250 mL Erlenmeyer flask and added with 5 mL of nitric acid and 5 ml of perchloric acid. The mixture was then heated at a temperature between 100-150 °C until the solution was clear. The sample solution was then cooled and diluted to 50 mL with distilled water.

### 2.3 Determination of zink

Instrumen atomic absorption spectroscopy Analytik Jena® ContrAA30 (Analytik Jena AG, Germany) was operated with flame  $\text{C}_2\text{H}_2$  in water. Output pressure

acethylene was operated on 80-100 kPa, the air was operated on 300-600 kPa. The velocity of air to flame was operated on 50 L/hour for Cd. Burner type was used is burner with wide 100 mm and the high was operated in 6 mm. -10 mL of sample solution is poured in flask and

then measured the absorbance in wavelength 213,857 nm for Zn.

### 2.4 Determination of mercury

Determination of Hg in cream cosmetics was performed using mercury analyzer as used by Noviana et al. (2012). Instrument of mercury analyzer Lab Analyzer-254 (gMBH, Karsfield, Germany) was operated with argon or nitrogen pump. Light source is Electrodeless Discharge Lamp. The velocity of air was operated on 30 L/hour. A-10 mL of sample solution is poured in flask then measured the absorbance in wavelength 253.7 nm for Hg.

### 2.5 Method validation

Method validation was performed by assessing several analytical figures of merit according to International Conference on Harmonization (ICH, 1994), namely linearity and range, precision, limit of detection (LOD) and limit of quantification (LOQ), and accuracy.

## 3. Results and Discussions

Several parameters have been taken into account and evaluated for the validation of the analytical methods for quantitative determination of heavy metals in cream cosmetis, namely, range and linearity, the minimum detection limit, the minimum limit of quantification, accuracy, and precision. Linearity study was demonstrated by analyzing six different concentrations of zink and Hg. Accurately measured standard working solutions of zink 0.1, 0.2, 0.5, 0.6, 0.8, 1.0, and 1.2  $\mu\text{g}/\text{mL}$  and mercury of 0.05, 0.1, 0.25, 0.75, 1.0, 1.5, and 2.0  $\mu\text{g}/\text{L}$  were prepared. The corresponding linear regression equation obtained has correlation coefficient of 0.9972 (Zn) and 0.9996 (Hg). According to Eurachem (1998), the analytical was linier over certain concentration ranges if  $R^2$  obtained is higher than 0.995. The linearity parameter and range for Zn and Hg is compiled in Table 1.

Table 1. Linear regression data for the calibration curve of zink (Zn) and mercury (Hg)

Parameter	Zn	Hg
Linearity range	0.1 - 1.2 $\mu\text{g}/\text{mL}$	0.05 - 2.0 $\mu\text{g}/\text{L}$
$R^2$	0.9972	0.9996
Slope $\pm$ SD	0.1397 $\pm$ 0.0064	0.1905 $\pm$ 0.0281
Intercept $\pm$ SD	0.0092 $\pm$ 0.0008	0.0009 $\pm$ 0.0052
Confidence limit of slope	0.1324 - 0.1445	0.1667-0.2216
Confidence limit of intercept	- 0.0078 - -0.0062	- 0.0246 - -0.0073

The sensitivity of AAS-flame and mercury analyzer was assesed by determining limit of detection (LOD) and limit of quantification (LOQ). LOD is the lowest concentration of analyte that can be detected and reliably distinguished from zero, but not necessarily quantified (Gonzales and Herrador, 2007), while LOQ is defined as the lowest concentration of analyte that can be determined quantitatively with an acceptable level of

Table 2. Precision studies data for determination of Zn and Hg.

Analyte	Day	Introduced analyte concentration ( $\mu\text{g/mL}$ )	Calculated analyte concentration ( $\mu\text{g/L}$ )	RSD (%)	
				Intraday	Interday
Zn (Zink)	1	0.20	$0.22 \pm 0.02$	10.78	
	2	0.20	$0.19 \pm 0.02$	8.67	9.89
	3	0.20	$0.22 \pm 0.02$	10.22	
Hg (Mercury)	1	1.00	$1.04 \pm 0.13$	12.69	
	2	1.00	$1.34 \pm 0.05$	3.79	7.17
	3	1.00	$1.27 \pm 0.06$	5.03	

Table 3. Accuracy studies data for analysis of Zink and Mercury

Analyte	Introduced analyte concentration ( $\mu\text{g/mL}$ )	Calculated analyte concentration ( $\mu\text{g/L}$ )	Recovery percentage (%)
Zn	0.20	0.20	101.31
	0.40	0.39	95.26
	0.60	0.52	84.23
Hg	0.50	0.33	64.15
	1.00	0.87	84.11
	1.50	1.33	87.70

Table 4. The results of Zn and Hg in cream cosmetics obtained from Yogyakarta.

Sample code	Zn ( $\mu\text{g/g cream}$ )	Hg ( $\text{ng/g cream}$ )
1	nd	10.12
2	2.17	12.59
3	nd	8.64
4	1.93	12.24
5	2.19	12.03

nd = not detected

precision and accuracy (Gonzales and Herrador, 2007; IUPAC, 1998). In order to determine LOD and LOQ, ten blank samples were measured. LOD and LOQ were calculated as  $3.3 \text{ SD}/b$  and  $10 \text{ SD}/b$ , respectively, where SD is the standard deviation of analytical responses and b is the slope of calibration curve. LOD and LOQ were found to be  $2.8614 \mu\text{g/g sample}$  and  $9.5281 \mu\text{g/g sample}$  respectively (for Zn using AAS-flame) and to be  $2.8614 \mu\text{g/kg sample}$  and  $1.5827 \mu\text{g/kg sample}$ , respectively (Hg using mercury analyzer).

Precision is usually measured as relative standard deviation (RSD) of a set of data (concentration in this study). Precision of the zinc and Hg was checked in order to show if instrument response to Zn standard solution was always reproducible (the same over different parameters). This parameter takes into account only the error coming from the operating system and not the error attributable to sample handling and preparation (Ertasa and Tezel, 2004). In order to assess the analytical method precision, measurements were done under conditions of repeatability and intermediate precision. Repeatability of the method was evaluated from the analysis 10 blank sample solutions fortified with  $0.2 \mu\text{g/mL}$  zinc standard and  $1.0 \mu\text{g/L}$  mercury standard, under the similar conditions (day, analyst, instrument, sample). The RSD values obtained were 8.67% (Zn) and 12.69% (Hg). Furthermore, the intermediate precision was evaluated

by performing 10 measurement with three different days. The RSD values obtained during the intermediate precision was 9.89% (Zn) and 7.17% (Hg). The results of precision studies were compiled in Table 2. According to RSD Horwitsch function (Gonzales and Herrador, 2007), the maximum RSD values acceptable for the level analyte of  $1 \mu\text{g/mL}$  is 16 %. Therefore, it can be stated that the developed method exhibited a good precision. The accuracy of the analytical method was determined by calculating recoveries of zinc and Hg. To ensure the accuracy of the analytical method, the recovery studies were carried out by adding a known quantity of analyte with preanalyzed by the proposed method. To check the accuracy of analytical method, the recovery studies were performed in order to confirm the losses of Zn and Hg or contamination during sample preparation as well as matrix interferences during the measurement step (Ertasa and Tezel, 2004). According to ICH (1994) for the determination of the recovery, the spiking technique was used, i.e the known concentration of Zn and Hg solution were added to cream cosmetics, and the resulting spiked samples were measured, calculated, and compared to the known value of Zn and Hg solution added. All analytical steps were performed in three replicates with three different levels of Zn concentration. The recovery values for accuracy studies of cream cosmetic samples spiked with different level of Zn and Hg were shown in Table 3. For the analyte level

of approximately 1 µg/mL, a recovery range of 80-110 % was acceptable (Taveniers *et al.*, 2004). Therefore, the developed method was accurate for quantification of zinc and Hg in cream cosmetics.

The developed method was further used for determination of Zn and Hg in some cream products. The levels of Zn and Hg as determined by AAS-flame and mercury analyzer were compiled in Table 4. Food and Drug Administration, Republic of Indonesia stipulated that the maximum level of Hg in cosmetics cream is 1 ppm. From Table 4, it is known that Hg level is lower than that required by regulatory agency.

#### 4. Conclusion

The atomic absorption spectroscopy using flame atomization and mercury analyzer were successfully used for quantitative analysis of Zn and Hg in cream cosmetics, respectively. The developed methods meet the acceptance criteria of validation parameters according to Internal Conference on Harmonization (ICH).

#### 5. Acknowledgement

The authors thank to Research and Testing Laboratory Gadjah Mada University (LPPT UGM) for financial support and facilities which make this study possible.

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