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Validation and quantitative analysis of cadmium and lead in snake fruit by flame atomic absorption spectrophotometry

[1,2*]Eka, N., [1]Astuti, [2,3]Retno, S. and [1,2,3]Rohman, A.

[1]*Intergrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta, Indonesia*
[2]*Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281, Indonesia*
[3]*Research Center of Halal Products, Gadjah Mada University, Yogyakarta 55281, Indonesia.*

Abstract: Snake fruit or known as *Salak* is one of the favorite fruits which is consumed not only in Indonesia but also in the worldwide. The fruit may be contaminated with heavy toxic metals like cadmium (Cd) and lead (Pb). As a consequence, the validation of analytical methods for determination of these heavy metals is continuously developed. Flame Atomic Absorption Spectrophotometer (FAAS) for determination of Cd and Pb in snake fruit has been validated. The developed method was linear over the concentration ranges of 0,01 -1,0 µg/mL (Cd) and 0,10 – 2,00 µg/mL (Pb). The correlation coefficient (r) obtained for both regressions is higher than 0.99. Limit of detection (LOD) of Cd and Pb were found to be 0,004 µg/mL and 0,04 µg/mL respectively, while limit of quantitation (LOQ) were found to be 0,01 µg/mL (Cd) and 0,1 µg/mL (Pb), respectively. This method was accurate and precise enough for determination of these heavy metals in snake fruit sample which were indicated by the acceptable recovery and low values of relative standard deviation. The developed method has been successfully used for determination of Cd and Pb levels in snake fruit commercially available in the markets.

Keywords: Method validation, cadmium, lead, FAAS, snake fruit.

Introduction

As part of human nutrition sources, fruits are consumed by all people in the worldwide. Besides their taste which make people love them, fruits contain various active chemical constituents capable of promoting human health. Snake fruit (*Salacca zalacca*), also locally known as "salak", is one of the favorite fruits for Indonesian people. "Pondoh" cultivar which was grown originally in Yogyakarta province is more popular (compared with other cultivars) among local Indonesian consumers due to the intensity of its aroma even before full maturation (Supriyadi *et al.*, 2002). Snake fruit also contains high comparable quantities of basic nutritional compounds (fibers, proteins, fats and carbohydrates) and possesses high level of antioxidant and proliferative activities (Gorinstein *et al.*, 2009). Previous study showed that snake fruit positively affected plasma lipid levels in rats fed cholesterol-containing diets (Leontowicz *et al*., 2007).

Heavy toxic metals can be accumulated in fruits with various concentrations depending on harvesting sites of fruits (Wagner, 1993). Cadmium (Cd) and lead (Pb) are two heavy metals which are harmful for human health. Cd has been reported to exert nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic effect (Kumar and Singh, 2010). Cadmium stimulates free radical production initiating various pathological conditions in humans (Waisberg *et al.*, 2003). Furthermore, Pb has toxic effects on the nervous system contributing to the permeability damage of the blood-brain barrier (Ruan and Gu, 1999). The excessive exposure of Pb may also result in end-stage renal disease (Weeden, 1982). As a consequence, the scientists continuously develop analytical method to determine Pb and Pb levels in various food matrixs, including fruits.

Atomic absorption spectrophotometry (AAS) is one of the most known techniques for determining heavy metals including Cd and Pb (Caldas and Machado, 2004; Garcia-Rico *et al.,* 2007). Cd and Pb can also be determined using spectro-polarimetric titrimetric method (Palma and Pearson, 1970), coupled plasma optical emission spectrometry (Froes *et al.*, 2009), near-infrared spectroscopy (Li *et al*., 2011) and visible spectrophotometry (Hashem, 2002). The presences of Cd and Pb in various samples such as in traditional herbs (Caldas and Machado, 2004) and supplements (Garcia-Rico *et al*., 2007) have been

*Corresponding author.
Email: *ekanoviana.91@gmail.com*

reported. However, using literature review, there was no available report related to the determination of Cd and Pb in snake fruit. Therefore, in this study, we developed and validated fast and reliable analytical technique of Flame Atomic Absorption Spectrophotometry (FAAS) for quantitative analysis of Cd and Pb in snake fruit.

Materials and Methods

Materials

Snake fruits were collected from traditional markets in Yogyakarta. Cadmium (Cd) and lead (Pb) standard solution (1000 mg/L) was purchased from Merck (Darmstadt, Germany) in the form of $Cd(NO_3)_2$ and $Pb(NO_3)_2$. All reagents and solvents used were of pro analysis (p.a) grade. Destilled and deionized water was used as solvent. All glassware used were soaked in detergent solution, followed by rinsing with distilled water. Flame Atomic Absorption Spectrophotometry (FAAS) of Analytik Jena® ContrAA 300 (Jena, Germany) was used for measuring the analytical response. The analytical balance used has sensitivity of 0.1 mg.

Digestion procedure

One kilogram of fresh snake fruit was peeled and cut into small pieces. This sample was subsequently subjected to digestion process. Digestion procedure was carried out according to Voegborlo and Akagi (2007) with slight modification. An approximately of 5 g of snake fruit sample was accurately weighed into 100 ml Erlenmeyer flask and added with 10 mL of the oxidizing reagent mixture composed of nitric acid– perchloric acid (1:1 v/v). The mixture was subsequently heated at a temperature between 100-110°C until the solution was clear. The sample solution was then cooled and diluted to 25 ml in volumetric flask with distilled water.

Determination of Cd and Pb using FAAS

FAAS instrument Analytik Jena® ContrAA 300 was operated under the following flame parameters: burner gas: C_2H_2/air (50 L/h and 65 L/h for Cd and Pb respectively); burner type: 100 mm, and burner height: 6 mm. Acetylene output pressure was adjusted at 80-100 kPa while air pressure was set at 300-600 kPa. Ten milliliters sample solution was then introduced to the sample tube and analyzed at wavelengths of 228,8 nm (Cd) and 217,0 nm (Pb).

Method validation

Method validation was assessed by determining several analytical figures of merit according to International Conference on Harmonization (ICH, 1994), namely linearity and range, precision, sensitivity which is expressed by determination of limit of detection (LOD) and limit of quantitation (LOQ), and accuracy.

Table 1. Linear regression data of Cadmium and Lead calibration curves

Parameters	Cd	Pb
Linear range	0,01-1,00 µg/mL	0,10-2,00 µg/mL
R^2	0,9991	0,9998
Slope ± SD	0,1315±0,0039	0,0209±0,0003
Intercept ± SD	0,0017±0,0019	0,0006±0,0004
Confidence limit of slope*	0,1276-0,1354	0,0206-0,0213
Confidence limit of intercept*	-0,0002-0,0036	0,0002-0,0010

* 95% confidence limit

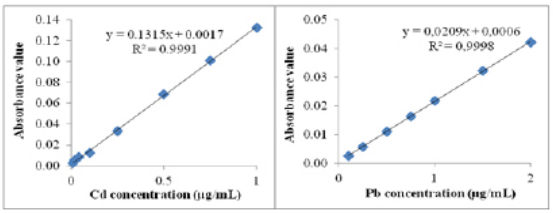

Figure 1. The relationship between concentration (x-axis) and the corresponding absorbance values (y-axis) of Cd and Pb

Results and Discussions

The validity of the developed method was evaluated by establishing some analytical figures of merit for determination of Cd and Pb in snake fruit.

Linearity

The linearity of response was assessed by plotting the absorbance values (y-axis) (of standard solutions of Cd and Pb versus its final concentration (x-axis). The concentration ranges used were 0,01-1,00 µg/mL and 0,10-2,00 µg/mL for Cd and Pb, respectively. A linear relationship existed for both regression equations with good coefficient of determination (R^2) (Table 1). According to Eurachem guide (1998), the analytical response was linear over certain concentration ranges if the R^2 value obtained is higher than 0.995. Figure 1 revealed the scatter plot for the relationship between the concentrations of Pb and Cd (x-axis) and the corresponding absorbance values (y-axis).

Determination of LOD and LOQ values

Sensitivity of the proposed method was performed by computing limit of detection (LOD) and limit of quantitation (LOQ). In order to determine LOD and LOQ, ten blank samples were measured. LOD and LOQ were calculated as 3.3SD/b and 10SD/b respectively, where SD is the standard deviation of analytical responses and b is the slope of calibration curve. When the sample blanks cannot produce any response, ten independent sample blanks fortified at

Table 2. Precision studies data

Cadmium				
day	Introduced analyte conc. (µg/mL)	Calculated analyte conc. (µg/mL)	RSD (%)	
			Repeatability	Intermediet precision
1	0,65	0,634±0,009	1,36	
2	0,65	0,624±0,007	1,05	1,33
3	0,65	0,632±0,007	1,10	
Lead				
day	Introduced analyte conc. (µg/mL)	Calculated analyte conc. (µg/mL)	RSD (%)	
			Repeatability	Intermediet precision
1	0,8	0,757±0,046	6,03	
2	0,8	0,748±0,050	6,74	6,35
3	0,8	0,744±0,051	6,84	

Table 3. Accuracy studies data

Cadmium			
Actual concentration (µg/mL)	Calculated concentration (µg/mL)	Recovery percentage	RSD (%)
0,2	0,206±0,000	103,24±0,16	0,15
0,5	0,483±0,001	96,62±0,10	0,10
1,0	0,954±0,003	95,39±0,33	0,35
Lead			
Actual concentration (µg/mL)	Calculated concentration (µg/mL)	Recovery percentage	RSD (%)
0,2	0,175±0,004	87,32±1,81	2,07
0,5	0,463±0,008	92,54±1,54	1,66
1,0	0,879±0,010	87,94±0,99	1,12

Table 4. Cadmium and Lead content in marketed snake fruits

Sample	Cd (µg/mL)	Pb (µg/mL)
Salak1	nd*	nd*
Salak2	0,060±0,004	nd*
Salak3	0,087±0,003	nd*
Salak4	0,071±0,001	nd*
Salak5	0,102±0,004	nd*
Salak6	0,074±0,005	nd*
Salak7	0,077±0,002	nd*

*nd: not detected in sampel

the lowest acceptable concentration of the analyte are measured (Gonzales and Herrador, 2007). LOD were found to be 0.004 µg/mL and 0.04 µg/mL for cadmium and lead, respectively. Meanwhile, the LOQ values found were of 0.01 µg/mL (Cd) and 0.1 µg/mL (Pb).

Precision

Precision is usually measured as relative standard deviation (RSD) of a set of data. The analytical precision was determined by assessing the reproducibility of instrument response to analyte. The measurements were done under the conditions of repeatability and intermediate precision (different day of measurement). Repeatability was evaluated by measuring 10 blank sample solutions fortified with the standard solutions of Cd (0.65 µg/mL) and Pb (0.8 µg/mL) under similar conditions (day, analyst, instrument, sample). The RSD values obtained were 1.36% and 6.03% for Cd and Pb, respectively. Furthermore, the intermediate precision was evaluated by performing 10 measurements with three different days. The RSD values obtained during the intermediate precision were 1.33% (Cd) and 6.35% (Pb), as shown in Table 2. According to Horwitz, as cited from Gonzalez and Herrador (2007), the maximum RSD value acceptable for the analyte level of 1 µg/mL is 16 %. AOAC set the maximum acceptable RSD value at 11% for the same analyte level (Gonzalez and Herrador, 2007). Therefore, it can be stated that the proposed method showed good precision based on RSD values obtained.

Accuracy

In order to check the accuracy of analytical method, the recovery studies were performed in this study. These recovery studies were done to confirm the lack of analyte levels due to the losses or contamination during sample preparation, and matrix interferences during the measurement step (Ertas and Tezel, 2004). According to ICH (1994), the recovery determination was carried out by spiking technique, i.e the known concentration of standard solutions (Cd and Pb) were added to snake fruit, and the resulting spiked samples were measured, calculated, and compared to the known value of standard solutions added. All analytical steps were performed in three replicates with three different levels of analyte concentration, as suggested by ICH. The recovery values for accuracy studies were shown in Table 3. According previous published study (Huber, 1998), the acceptable recovery percentage range is 80-110% for the analyte level of 1 µg/mL. Therefore, the developed method was accurate for quantification of Cd and Pb in snake fruit.

Quantification of Cd and Pb in snake fruit

The levels of Cd and Pb in some marketed snake fruit samples were quantified using the developed method. The levels of Cd and Pb in snake fruit were shown in Table 4. The levels of Cd from six marketed snake fruit samples were found to be 0.060±0.004; 0 .087±0.003;0.071±0.001;0.102±0.004; 0.074±0.005; and 0.077±0.002 µg/mL, while the levels of Pb were not detected in all studied samples. According to Indonesian government regulation, the maximum levels of Cd and Pb permissible in fruit were 0.2 µg/g and 0.5 µg/g, respectively, (Indonesian National standard, 2009). The results showed that the levels of Cd and Pb in commercially snake fruit samples were lower than those specified in Indonesian standard.

Conclusion

Analytical method development of Cd and Pb in snake fruit using FAAS has been developed. Evaluation of analytical method parameters including linearity, sensitivity, precision and accuracy showed acceptable results. Furthermore, the developed method can be successfully used for determination of Cd and Pb in snake fruits available in the markets. The levels of Pb and Cd reported were lower than those required by Indonesian government.

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