Asian Journal of
Agricultural
Research

CrossMark
← click for updates

Validation of Atomic Absorption Spectrophotometry and Mercury Analyzer for Analysis of Cadmium, Lead and Mercury in Red Chili Pepper

[1]EllsyaAngeline Rawar and [1,2]Abdul Rohman

[1]Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281, Indonesia
[2]Integrated Laboratory of Research and Testing, Gadjah Mada University, Yogyakarta, 55281, Indonesia

Corresponding Author: Abdul Rohman, Integrated Laboratory of Research and Testing, Gadjah Mada University, Yogyakarta, 55281, Indonesia

ABSTRACT

A Flame-Atomic Absorption Spectrophotometry (AAS-flame) for the quantification of cadmium (Cd) and lead (Pb) and mercury analyzer for quantitative analysis of mercury (Hg) in red chili pepper (*Capsicum annum* L.) were developed and validated. The method was validated for some parameters, namely linearity and range, precision, accuracy, Limit of Detection (LoD) and Limit of Quantification (LoQ), according to International Conference on Harmonization (ICH). The standard curves were linear over the concentration ranges of 0.1-1.4 µg mL^{-1} (Cd), 0.10-1.80 µg mL^{-1} (Pb) and 0.5-20 µg mL^{-1} with correlation coefficient (r) values >0.99. The detection limits obtained were 0.022 µg mL^{-1}, 0.0594 µg mL^{-1} and 1.77 µg mL^{-1} for Cd, Pb and Hg, respectively. The quantification limits obtained were 0.075 µg mL^{-1} (Cd), 0.1981 µg mL^{-1} (Cd) and 5.91 µg mL^{-1} (Hg). The Relative Standard Deviation (RSD) values found for its intra-day and interday precision were lower than those required by RSD Horwitz. The mean recovery percentage was 97.98-101.9133%.The validated methods were successfully used for determination of Cd, Pb and Hg in red chili pepper.

Key words: Cadmium, lead, mercury, atomic absorption spectroscopy, mercury analyzer, red chili pepper

INTRODUCTION

Atomic Absorption Spectrophotometry (AAS) is the method of choice for determination heavy metals contained in food samples (Vogel, 2001). The application of chemicals on farming can cause pollution of soils. Heavy metals cause a long-term risk on environmental and human health (Wong *et al.*, 2002). Besides, they can accumulate to toxic levels (Sharma *et al.*, 2007) which can contaminate soils in which some plant grow. Heavy metal such as cadmium (Cd), lead (Pb) and mercury (Hg) are hazardous metals because of its harmful effects on environment, plants, animal and human (Waisberg *et al.*, 2003).

Cadmium (Cd) can induce severe alterations in various organs and tissues following either acute or chronic exposure (Nordberg *et al.*, 1985). Cadmium toxicity may be manifested by a variety of syndromes and effects, including renal dysfunction, hypertension, hepatic injury, lung damage and teratogenic effects (Oehme, 1978). A number of Cd-induced effects include deterioration of cell-cell adhesion, DNA-related processes, cell signaling and energy metabolism, which imply

that Cd acts on the different molecular targets in human organism (Shimoda *et al.*, 2001; Ivanoviene *et al.*, 2004). Lead can accumulate in aquatic biomass and then is concentrated and passed up the food chain to human consumers. Lead (Pb) can damage the brain, the central nervous system, kidneys, liver and the reproductive system (Ademoroti, 1996). Maenwhile, mercury cause adverse effects on the nervous systems and renal. They can cross the placental barrier so that mercury is potential toxic effects on the fetus (Tong *et al.*, 2000). Mercury gets into the body through the respiratory and gastrointestinal tract and is distributed into the liver and kidney. It is also accumulated in nervous tissue. The alkylated mercury accumulates in the Central Nervous System (CNS) tissue. It is toxic to the glial cells, mostly Schwann cell production of myelin, neuropsychotic, severe developmental CNS abnormalities in foetus and prolonged action potentials (Mckinney and Rogers, 1992).

Red chili peppers are parts of human diet. They are used throughout the world as spices and medicines. One hundred grams of them has 11000 IU of vitamin A, 0.7 mg of vitamin E and 240 mg of vitamin C (Govindarajan, 1986). They contain polyphenol compounds, which include flavonols, hydroxycinnamates and flavones. Red chili peppers are known for their rich antioxidant contents such as vitamin C and vitamin E (Simonne *et al.*, 1997). Capsaicinoids, the red pigment on the red chili pepper have two modes of action. First, the sensation of heat which may help sore muscles and arthritic joints feel better. Second, the depletion of substance P which reduces the pain sensation in the exposed area. Anticoagulant in peppers help prevent the blood clots that cause heart attacks (Andrews, 1995). Red chili pepper has long been used to inhibit pain messenger and alleviate the pain of arthritis, headaches, burns and neuralgia. Red chili peppers can be contaminated with Cd, Pb and Hg, therefore the levels of these heavy metals must be determined for the quality assessment of red chili peppers.

The most known techniques for analysis of heavy metals including Cd Pb and Hg is Atomic Absorption Spectrophotometry (AAS) using flame and cold vapor atomizer (Caldas and Machado, 2004; Garcia-Rico *et al.*, 2007). The heavy metals of Cd and Pb can also be analyzed 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). Meanwhile, the presence of mercury (Hg) in some food matrixes has been determined using cold vapor atomic absorption spectrophotometry (CV-AAS) (Rahimi *et al.*, 2010; Millan *et al.*, 2006; Voegborlo and Akagi, 2007). Using literature searching, there is no reports regarding the use of AAS-flame and mercury analyzer for determination of Cd, Pb and Hg in red chili peppers. The purpose of this study is to validate and used AAS and Hg-analyzer for determination of Cd, Pb and Hg in red chili peppers (*Capsicum annum* L.) distributed in some markets in Yogyakarta, Indonesia.

MATERIALS AND METHODS

Materials: Red chili peppers were obtained from traditional markets in Yogyakarta. The standard solution of Cadmium (Cd), lead (Pb) and mercury (Hg) were supplied by Sigma Aldrich (Steinheim, Swiss). The other reagents were supplied by Merck (Darmstadt, Germany). All reagents were of analytical grade. The water used as solvent was distilled and deionized waters. All glass wares used were cleaned by soaking them in detergent solution, then rinsing them with distilled water.

Digestion procedure: The digestion procedure was carried out according to Voegborlo and Akagi (2007). An approximately of 1 g of red chili pepper was accurately weighed into 250 mL Erlenmeyer flask and added with 8 mL of nitric acid (HNO_3) and 4 mL of perchloric acid ($HClO_4$). The mixture was then heated at a temperature between 150-250°C until the solution was clear. The sample solution was then cooled and diluted to 25 mL with distilled water.

Determination of cadmium: Instrument of atomic absorption spectrophotometer Analytik Jena® ContrAA30 (Analytik Jena AG, Germany) was operated with flame C_2H_2 in water. Output pressure of acetylene was operated on 80-100 kPa, the pressure of air was operated on 300-600 kPa. The velocity of air to flame was operated at 50 L h^{-1} for Cd and Pb. Burner type used is burner with wide 100 mm and high in 6 mm. A-10.0 mL sample solution is poured in flask and then the absorbance was measured in wave lengths of 228.8 nm for Cd and 217.0 nm for Pb.

Determination of Hg: The samples were transferred into a reaction flask and 0.5 mL of Tin (II) chloride 10% (w/v) in HCl 1 M as reducing agent. The bottle was then connected to the reaction unit of mercury analyzer (Lab Analyzer 254) and measurement was started. The mercury was quickly stripped from the reaction flask. After 60-80 sec, the end of the analysis was acoustically anounced and the measurenment result (absorbance) was displayed. The absorbance values were used for the calculation of analyte contents in red chili pepper samples.

Method validation: Method validation was performed by assessing several analytical figures of merit according to ICH. (1994), namely linearity and range, precision, Limit of Detection (LoD), Limit of Quantification (LoQ) and accuracy.

RESULTS AND DISCUSSION

Several parameters have been taken into account and evaluated during the validation of the analytical methods for quantitative analysis of heavy metals (Cd and Pb using AAS-flame as well as Hg using mercury analyzer) in red chili peppers, namely, range and linearity, the minimum detection limit (LoD), the minimum Limit of Quantification (LoQ), accuracy and precision.

Linearity and range: Linearity was demonstrated by analyzing six different concentrations of Cd, Pb and Hg. The accurately measured standard working solutions of cadmium 0.1, 0.2, 0.4, 0.8, 1.0, 1.2, 1.4 µg mL^{-1}; 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.7, 1.8 µg mL^{-1} (Pb) and 0.5, 1.0, 2.5, 7.5, 10.0, 15.0 and 20.0 µg L^{-1} (Hg) were used for evaluation of linearity and range.The coefficient correlation (r) values obtained corresponding to linear regression equation were 0.9962 (Cd), 0.9981 (Pb) and 0.9956 (Hg). According to Eurachem (1998), the analytical method was linier over certain concentration ranges if rvalues obtained are higher than 0.995. Table 1 compiled the linearity and range parameters for analysis of Cd, Pb and Hg.

Table 1: Linear regression data for the collaboration curve of Cd, Pb and Hg

Parameters	Cd	Pb	Hg
Linearity range	0.1-1.4 µg mL^{-1}	0.1-1.8 µg mL^{-1}	0.5-20.0 µg mL^{-1}
R^2	0.9962	0.9981	0.9956
Slope±SD	0.1123±0.00119	0.0222±0.0002	0.0164±0.0004
Intercept±SD	0.0092±0.00047	0.0004±0.0003	-0.0016±0.0001
Confidence limit of slope	0.1111-0.1135	0.0221-0.0224	0.0160-0.0168
Confidence limit of intercept	0.0087-0.0097	0.0001-0.0008	-0.0017-(-0.0015)

Table 2: Precision studies data for analysis of Cd and Pb using AAS-flame and analysis Cd using mercury analyzer

Analyte	Day	Level of analyte used (μg mL^{-1})	Calculated analyte concentration (μg L^{-1})	RSD (%)	
				Intraday	Interday
Cd	1	0.40	0.39±0.01	2.04	2.11
	2	0.40	0.38±0.01	1.83	
	3	0.40	0.39±0.01	2.45	
Pb	1	0.60	0.60±0.01	1.12	3.09
	2	0.60	0.58±0.02	4.08	
	3	0.60	0.58±0.02	4.07	
Hg	1	10.00	11.30±1.38	12.22	11.83
	2	10.00	11.30±1.11	9.86	
	3	10.00	10.61±1.42	13.40	

Sensitivity assay: Sensitivity of analytical methods can be described by values of Limit of Detection (LoD) and Limit of Quantification (LoQ). The LoD is the lowest concentration of analyte that can be detected and reliably distinguished from zero, but not necessarily quantified (Gonzalez and Herrador, 2007). The LoQ can be defined as the lowest concentration of analyte that can be determined quantitatively with an acceptable level of precision (Gonzalez and Herrador, 2007). In order to get LoD and LoQ values, ten blank samples were measured. The values of LoD and LoQ were calculated as:

$$LoD = 3.3 \text{ standard deviation (SD)}/b$$

$$LoQ = 10 \text{ standard deviation (SD)}/b$$

where, standard deviation is SD for blank responses and b is slope of regression equation at low concentration level. The values of LoD and LoQ obtained were 0.022 and 0.075 μg mL^{-1} (Cd), 0.0594 and 0.1981 μg mL^{-1} (Pb), as well as 1.77 and 5.91 μg mL^{-1} (Hg).

Precision: Precision of an analytical method is usually measured as Relative Standard Deviation (RSD) of a set of data (concentration in this study). Precision of the analytical method for analysis Cd and Pb (AAS-flame) and Hg (using mercury analyzer) was checked in order to show the reproducibility of responses. 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, 2005).

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 0.4 μg mL^{-1} (Cd), 3 μg mL^{-1} (Pb) and 3 μg L^{-1} (Hg) under the similar conditions (day, analyst, instrument, sample). The RSD values obtained were 2.04% (Cd), 1.12% (Pb) and 9.86% (Hg). Furthermore, the intermediate precision was evaluated by performing 10 measurements with three different days. The RSD values obtained during the intermediate precision were 2.11% (Cd), 3.09% (Pb) and 11.83% (Hg), as seen in Table 2. According to RSD Horwitch, as cited from Gonzalez and Herrador (2007), the maximum RSD values acceptable for the level analyte of 1 μg mL^{-1} is 16%. Therefore, it can be stated that the developed method exhibited a good precision.

Accuracy: The accuracy of the method was determined by calculating recoveries of Cd, Pb and Hg. To ensure the accuracy of the analytical method, the recovery studies were carried out by adding

Table 3: Recovery values for accuracy studies of Cd and Pb determined using AAS-flame and Hg determined using mercury analyzer

Analytes	Introduced analyte concentration ($\mu g\ mL^{-1}$)	Calculated analyte concentration ($\mu g\ L^{-1}$)	Recovery percentage (%)
Cd	0.20	0.2020	101.02
	0.40	0.4334	108.36
	0.60	0.5781	96.36
Pb	0.20	0.2278	101.34
	0.60	0.5966	91.20
	0.80	0.7499	101.21
Hg	1.25	1.266	101.34
	3.00	2.736	91.20
	5.00	5.061	101.21

Table 4: Content of Cd, Pb and Hg in red chili pepper (*Capsicum annum* L.) samples obtained from several market in Yogyakarta

Samples	Cd levels ($\mu g\ g^{-1}$)	Pb levels ($\mu g\ g^{-1}$)	Hg levels ($\mu g\ kg^{-1}$)
1	Not detected	Not detected	15.98
2	Not detected	Not detected	14.18
3	Not detected	Not detected	12.44
4	Not detected	Not detected	15.76
5	Not detected	Not detected	13.51
6	Not detected	Not detected	11.67
7	Not detected	Not detected	231.41
8	Not detected	Not detected	13.26
9	Not detected	Not detected	15.28
10	Not detected	Not detected	193.57

a known quantity of Cd, Pb and Hg into samples by the proposed method. To check the accuracy of analytical method, the recovery studies were performed in order to confirm the losses of Cd, Pb and Hg or contamination during sample preparation and matrix interferences during the measurement step (Ertasa and Tezel, 2005). According to ICH (1994) for the determination of the recoveries, the spiking technique was used, i.e., the known concentration of standard solutions of Cd, Pb and Hg were added to *Capsicum annum* L. and the resulting spiked samples were measured, calculated and compared to the known values of standard solutions of Cd, Pb and Hg added. All analytical steps were performed in three replicates with three different levels of Cd, Pb and Hg concentration. The recovery values for accuracy studies of samples spiked with different level of Cd, Pb and Hg were compiled in Table 3. For the analyte level of approximately $1\ \mu g\ mL^{-1}$, a recovery range of 80-110% was acceptable (Taverniers *et al.*, 2004). Therefore, the developed method was accurate for quantification of Cd, Pb and Hg in red chili peppers.

Determination of Cd, Pb and Hg in red chili pepper samples: The developed and validated method was applied for the determination of Cd, Pb and Hg content in *Capsicum annum* L. The results were shown in Table 4. The heavy metals of Cd and Pb were not detected in all evaluated samples. The mercury level in samples was found in the range of $9.74\text{-}232.58\ \mu g\ kg^{-1}$.

CONCLUSION

Atomic Absorption Spectrophotometry (AAS) for determination of Cd and Pb as well as mercury analyzer for analysis of Hg has been validated. The validation parameters of both methods fit the criteria required by International Conference on Harmonization (ICH). The validated methods was succesufully usedfor quantitative analysis of Cd, Pb and Hg in red chili pepper samples. The heavy metals of Cd and Pb were not detected in all evaluated samples, while Hg is present in the range of $9.74\text{-}232.58\ \mu g\ kg^{-1}$.

REFERENCES

Ademoroti, C.M.A., 1996. Environmental Chemistry and Toxicology. 1st Edn., Foludex Press Ltd., Ibadan, Nigeria.

Andrews, J., 1995. Peppers: The Domesticated Capsicums. University of Texas Press, Austin, Texas, ISBN-13: 9780292704671, Pages: 186.

Caldas, E.D. and L.L. Machado, 2004. Cadmium, mercury and lead in medicinal herbs in Brazil. Food Chem. Toxicol., 42: 599-603.

Eurachem, 1998. The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics. LGC, United Kingdom.

Ertasa, O.S. and H. Tezel, 2005. A validated cold vapour-AAS method for determining cadmium in human red blood cells. J. Pharm. Biomed. Anal., 36: 893-897.

Froes, R.E.S., W.B. Neto, N.O.C. Silva, R.L.P. Naveira, C.C. Nascentes and J.B.B. da Silva, 2009. Multivariate optimization by exploratory analysis applied to the determination of microelements in fruit juice by inductively coupled plasma optical emission spectrometry. Spectrochimica Acta Part B: Atomic Spectrosc., 64: 619-622.

Garcia-Rico, G., J. Leyva-Perez and M.E. Jara-Marini, 2007. Content and daily intake of copper, zinc, lead, cadmium and mercury from dietary supplements in Mexico. Food Chem. Toxicol., 45: 1599-1605.

Gonzalez, A.G. and M.A. Herrador, 2007. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. Trends Anal. Chem., 26: 227-238.

Govindarajan, V.S., 1986. *Capsicum* production technology chemistry and quality part IIBProcessed products, standards, world production. Crit. Rev. Food Nutr., 23: 207-288.

Hashem, E.Y., 2002. Spectrophotometric studies on the simultaneous determination of cadmium and mercury with 4- (2-pyridylazo) -resorcinol. Spectrochimica Acta Part A: Mol. Biomol. Spectrosc., 58: 1401-1410.

ICH., 1994. Validation of analytical procedures: Text and methodology. International Conference on Harmonisation (ICH), June, 2014, San Diego CA.

Ivanoviene, L., I. Staneviciene, V. Lesauskaite, I. Sadauskiene and L. Ivanov, 2004. Activities of tRNALeu and leucyl-tRNA synthetase and programmed cell death in the liver of mice under experimental cadmium poisoning. Trace Elem. Elec., 21: 180-184.

Li, J., Y. Zhang, W. Cai and X. Shao, 2011. Simultaneous determination of mercury, lead and cadmium ions in water using near-infrared spectroscopy with preconcentration by thiol-functionalized magnesium phyllosilicate clay. Talanta, 84: 679-683.

McKinney, J. and R. Rogers, 1992. Metal bioavailability. Environ. Sci. Technol., 26: 1298-1299.

Millan, R., R. Gamarra, T. Schmid, M.J. Sierra and A.J. Quejido *et al.*, 2006. Mercury content in vegetation and soils of the Almaden mining area (Spain). Sci. Total Environm., 368: 79-87.

Nordberg, G.F., T. Kjellstrom and M. Nordberg, 1985. Kinetics and Metabolism. In: Cadmium and Health: A Toxicological and Epidemiological Appraisal, Friberg, L., C.G. Elinder, T. Kjellstrom and G.F. Nordberg (Eds.). CRC Press, Boca Raton, FL., pp: 103-178.

Oehme, F.W., 1978. Toxicity of Heavy Metals in the Environment Part I. 1st Edn., Marcel Dekker, Inc., New York, ISBN: 0824767187.

Palma, R.J. and K.H. Pearson, 1970. A spectropolarimetric titrimetric method for the determination of cadmium, mercury, lead and bismuth. Anal. Chim. Acta, 49: 497-504.

Rahimi, E., M. Hajisalehi, H.R. Kazemeini, A. Chakeri and A. Khodabakhsh *et al.*, 2010. Analysis and determination of mercury, cadmium and lead in canned tuna fish marketed in Iran. Afr. J. Biotechnol., 9: 4938-4941.

Sharma, R.K., M. Agrawal and F.M. Marshall, 2007. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicol. Environ. Safe., 66: 258-266.

Shimoda, R., T. Nagamine, H. Takagi, M. Mori and M.P. Waalkes, 2001. Induction of apoptosis in cells by cadmium: Quantitative negative correlation between basal or induced metallothionein concentration and apoptotic rate. Toxicol. Sci., 64: 208-215.

Simonne, A.H., E.H. Simonne, R.R. Eitenmiller, H.A. Mills and N.R. Green, 1997. Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). J. Food Comp. Anal., 10: 299-311.

Taverniers, I., M. De Loose and E. Van Bockstaele, 2004. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. TrAC Trends Anal. Chem., 23: 535-552.

Tong, S., Y.E. von Schirnding and T. Prapamontol, 2000. Environmental lead exposure: A public health problem of global dimensions. Bull. World Health Organ., 78: 1068-1077.

Voegborlo, R.B. and H. Akagi, 2007. Determination of mercury in fish by cold vapour atomic absorption spectrometry using an automatic mercury analyzer. Food Chem., 100: 853-858.

Vogel, A.I., 2001. A Text-Book of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis. 5th Edn., Longman Sc Tech., New York, USA., pp: 80.

Waisberg, M., P. Joseph, B. Hale and D. Beyersmann, 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology, 192: 95-117.

Wong, S.C., X.D. Li, G. Zhang, S.H. Qi and Y.S. Min, 2002. Heavy metals in agricultural soils of the Pearl River Delta, South China. Environ. Pollut., 119: 33-44.