

Validation of mercury analyzer for determination of mercury in snake fruit

^{1,2*}Eka, N., ¹Heri, D. H., ¹Astuti and ^{1,2,3}Rohman, A.

¹Intergrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta, Indonesia

²Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia

³Halal Research Group, Gadjah Mada University, Yogyakarta 55281, Indonesia.

Abstract: Mercury analyzer, an instrument specifically designed for mercury (Hg) determination, has been developed and validated for quantification of Hg levels in Salak or snake fruit (*Salacca zalacca*) obtained in Yogyakarta. Some analytical figures of merit, namely linearity and range, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) have been evaluated. The developed method is linear over 0.5 – 20 µg/mL with coefficient of correlation (r) > 0.99. LOD and LOQ obtained were 0,03 µg/L and 0,1 µg/L respectively. This method was quite precise with the relative standard deviation (RSD) values found to be 9.73% and 10.41% for its intraday and interday precisions. These RSD values were lower than those required by Horwitz. The mean recovery percentage was 97.24% indicating that the developed method was accurate for Hg determination in snake fruit. Mercury levels in the marketed snake fruit samples were found in the range of 4.87-15.08 µg/Kg which were lower than maximum permissible limit set by Indonesian government regulation, as 30 µg/Kg.

Key words: Method validation, mercury, mercury analyzer, snake fruit

Introduction

Fruits are parts of human diet sources. They contain various active components which give beneficial effects for human health, such as antioxidant (Rohman *et al.*, 2010), anticarcinogenic (Hakkinen *et al.*, 1999), antimutagenic (Jayaprakasha *et al.*, 2007) and antibacterial (Kaur and Singh, 2008) agents. Previous epidemiological study showed that there was an inverse relationship between consuming fruits and coronary heart disease and cancer incidences (Rohman *et al.*, 2007; Tsai *et al.*, 2010).

The heavy metals can be accumulated with different concentrations depending on their harvesting sites in the fruits (Wagner, 1993). One of the hazardous heavy metals which can endanger human health is mercury (Hg). Mercury has been known to cause adverse effects on the renal and nervous systems and can cross the placental barrier, with potential toxic effects on the fetus (Tong *et al.*, 2000; Caldas and Machado, 2004). As a consequence, the researchers continuously developed analytical method to quantify Hg levels in some matrix. Analytical method validation is an important step in the quality assurance of quantitative analysis. According to ISO/IEC 17025 (2005), method validation intends to guarantee that the method meets the acceptable criteria.

Several analytical methods have been used for analysis of Hg in some food matrix such as cold vapor atomic absorption spectrophotometry (CV-

AAS) for Hg analysis in tuna fish (Rahimi *et al.*, 2010), in traditional medicinal herbs, (Caldas dan Machado, 2004), supplement formulations (García-Rico *et al.*, 2007) and in soil (Millan *et al.*, 2006). CV-AAS using automatic mercury analyzer has also reported for Hg determination in fish (Voegborlo and Akagi, 2007) and in selected fruit and mushroom (Chudzyn'ski *et al.*, 2011). However, there is no available report related to the application of Mercury analyzer for quantification of Hg in snake fruit. Snake fruit (*Salacca zalacca*) is one of favorite fruits for Yogyakarta people, therefore its quality control including the levels of heavy metal present in it is very essential. Therefore, in this study, we developed and validated mercury analyzer for quantification of Hg in snake fruit.

Materials and Methods

Materials

Snake fruits were collected from traditional markets in Yogyakarta. Mercury standard solution (1000 mg/L) was purchased from Sigma Aldrich (Steinheim, Swiss). The other reagents were obtained from Merck (Darmstadt, Germany). All reagents were pro analysis grade. Distilled and deionized water was used as solvent. All glassware used were soaked in detergent solution, followed by rinsing with distilled water. Mercury Instrument®

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

Lab Analyzer 254 (Karlsfield, Germany), equipped with electrodeless low-pressure mercury lamp (EDL) as UV source, long-life diaphragm pump and 23 cm-measuring cell made entirely of fused silica, was used. The measurement wavelength was 253.7 nm and the air flow was set at 30 L/h. The principal measurement of mercury analyzer was shown in figure 1. The analytical balance used has sensitivity of 0.1 mg.

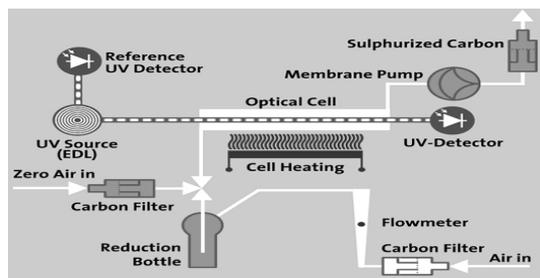


Figure 1. Schematic flow diagram of mercury analyzer

Digestion procedure

Digestion procedure was carried out according to Voegborlo and Akagi (2007) with slight modification. Briefly, an approximately of 5 g of homogenized snake fruit sample was accurately weighed into 100 ml Erlenmeyer flask and added with 10 mL of the mixture 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 with distilled water.

Determination of Hg

The sample was transferred into a reaction flask and 0.1 mL of Potassium permanganate 0.1 M was then added to the flask and the flask was shaken. One tenth milliliters of hydroxylammonium chloride 10% (w/v) was added to reduce excess permanganate. Then, 0.5 mL of Tin (II) chloride 10% (w/v) in HCl 1 M was subsequently added to reduce mercury contained in the sample. The bottle was then connected to the reaction unit of the Lab Analyzer 254 and measurement was started. The mercury was quickly stripped from the reaction flask. After 60 - 80 second, the end of the analysis was acoustically announced and the result (absorbance) measurement was displayed. The absorbance values were used for the calculation of analyte contents in snake fruit sample.

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,

sensitivity expressed by determination of limit of detection (LOD) and limit of quantification (LOQ), and accuracy.

Results and Discussions

In order to evaluate the validity of the proposed method, analytical performance characteristics for determination of mercury in snake fruit were estimated.

Linearity

Linearity of response was studied using mercury standard solutions containing 0.5, 1.0, 2.5, 7.5 10.0, 15.0 and 20.0 µg/L by plotting the absorbance values for each solution (y-axis) versus its final mercury concentration (x-axis). A linear relationship was obtained until 20.0 µg/L with a good coefficient of determination (R^2) (Table 1). The corresponding linear regression equation was $y = 0,034x - 0,009$. According to Eurachem (1998), the analytical was linier over certain concentration ranges if R^2 obtained is higher than 0.995.

Table1. Linear regression data for the calibration curve

| Parameters | |
|-------------------------------|----------------|
| Linearity range | 0.5-20 µg/L |
| R^2 | 0.9996 |
| Slope±SD | 0.034±0.009 |
| Intercept±SD | -0.009±0.001 |
| Confidence limit of slope | 0.033-0.035 |
| Confidence limit of intercept | -0.018(-0.001) |

LOD and LOQ

Limit of detection (LOD) is the lowest concentration of analyte that can be detected and reliably distinguished from zero, but not necessarily quantified (Gonzales and Herrador, 2007). Limit of quantitation (LOQ) is defined as the lowest concentration of analyte that can be determined quantitatively with an acceptable level of precision (Gonzalez and Herrador, 2007 cit IUPAC, 1998). 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. LOD and LOQ were found to be 0.03 µg/L and 0.10 µg/L respectively. This method was very sensitive for analyzing mercury content compared to the conventional atomic absorption spectroscopy whose LOD was found to be 0.16 µg/mL (Khopkar, 1990).

Precision

Precision is usually measured as relative standard deviation (RSD) of a set of data (concentration in this study). Precision of the mercury analyzer was checked in order to show if instrument response to Hg 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 of 10 blank sample solutions fortified with 3 µg/L Hg standard under the similar conditions (day, analyst, instrument, sample). The RSD value obtained was 9.73%. Furthermore, the intermediate precision was evaluated by performing 10 measurements with three different days. The RSD values obtained during the intermediate precision was 10.41% (Table 2). According to Horwitz, as cited from Gonzalez and Herrador (2007), the maximum RSD value acceptable for the analyte level of 10.0 µg/L is 32 %. Therefore, it can be stated that the developed method exhibited a good precision.

Accuracy

To check the accuracy of analytical method, the recovery studies were performed in order to confirm the lack of Hg losses or contamination during sample preparation and matrix interferences during the measurement step (Ertasa and Tezel, 2004). According to ICH (1998) for the determination of the recovery, the spiking technique was used, i.e the known concentration of Hg solution were added to snake fruit, and the resulting spiked samples were measured, calculated, and compared to the known value of Hg solution added. All analytical steps were performed in three replicates with three different levels of Hg concentration. The recovery values for accuracy studies of snake fruit samples spiked with different level of Hg was shown in Table 3. For the analyte level of approximately 10.0 µg/L, a recovery range of 60 – 115 % was acceptable (Taverniers *et al.*, 2004). Therefore, the developed method was accurate for quantification of mercury.

Hg content determination in snake fruit

Using the proposed method, the quantitation of Hg content in snake fruit was carried out. The results were shown in Table 4. The mercury level in samples was found in the range of 4.87-15.08 µg/

Kg. The maximum level of permissible Hg content in fruit was 30 µg/Kg according to Indonesian government regulation. These results showed that the mercury levels in marketed snake fruit samples were acceptable.

Table 2. Precision studies data

| day | Introduced analyte concentration (µg/L) | Calculated analyte concentration (µg/L) | RSD (%) | |
|-----|---|---|----------|----------|
| | | | Intraday | Interday |
| 1 | 3.0 | 2.95±0.29 | 9.73 | |
| 2 | 3.0 | 2.66±0.25 | 9.34 | 10.41 |
| 3 | 3.0 | 2.71±0.27 | 10.02 | |

Table 3. Accuracy studies data

| Introduced analyte concentration (µg/L) | Calculated analyte concentration (µg/L) | Recovery percentage | RSD (%) |
|---|---|---------------------|---------|
| 2.0 | 2.01±0.03 | 100.49±1.69 | 1.68 |
| 4.0 | 3.82±0.03 | 95.61±0.63 | 0.65 |
| 6.0 | 5.74±0.05 | 95.62±0.77 | 0.81 |

Table 4. Hg level determination in snake fruit

| Samples | Hg levels (µg/Kg) |
|---------|-------------------|
| Salak1 | 5.64±0.31 |
| Salak2 | 9.62±0.04 |
| Salak3 | 4.87±0.14 |
| Salak4 | 5.94±0.11 |
| Salak5 | 8.53±0.08 |
| Salak6 | 6.95±0.43 |
| Salak7 | 15.08±0.16 |

Conclusion

The mercury analyzer was successfully developed for quantitative analysis of Hg in snake fruits. The evaluated parameters for analytical method validation exhibited the acceptable values. The developed method was rapid, easy in sample preparation and inexpensive.

Acknowledgement

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

References

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

- Chudzyn'ski, K., Jarzyn'ska, G., Stefan'ska, A. and Falandysz, J. 2011. Mercury content and bio-concentration potential of Slippery Jack, *Suillus luteus*, mushroom. *Food Chemistry* 125: 986–990.
- Ertasa, O.S and Tezel, H. 2005. A validated cold vapour-AAS method for determining mercury in human red blood cells. *Journal of Pharmaceutical and Biomedical Analysis* 36: 893–897
- Eurachem, 1998, The fitness for purpose of analytical method: A laboratory guide to method validation and related topics, accessed from: <http://www.eurachem.org/guides/pdf,18/04/2011>
- Garcia-Rico, G., Leyva-Perez, J. and Jara-Marini, M.E. 2007. Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico. *Food and Chemical Toxicology* 45: 1599–1605.
- Gonzalez, A.G. and Herrador, M.A., 2007. A Practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. *Trends in Analytical Chemistry* 26: 227-238.
- Hakkinen, S., Heinonen, M., Karenlampi, S., Mykkanen, H., Ruuskanen, J., Torronen, R. 1999. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Research International* 32: 345–353.
- International Conference on Harmonisation (ICH), 1994, Validation of Analytical Procedures: Text and Methodology, accessed from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf, 19/04/2011.
- ISO/IEC 17025. 2005. General requirements for the competence of testing and calibration laboratories.
- Jayaprakasha, G.K., Negi, P.S., Jena, B.S., Mohan Rao, L.J. 2007. Antioxidant and antimutagenic activities of *Cinnamomum zeylanicum* fruit extracts. *Journal of Food Composition and Analysis* 20: 330-336.
- Kaur, G. and Singh, R.P. 2008. Antibacterial and membrane damaging activity of *Livistona chinensis* fruit extract. *Food and Chemical Toxicology* 46: 2429-2434.
- Khopkar, S.M. 1990. Basic Concept of Analytical Chemistry, UI Press, Jakarta.
- Millán, R. Gamarra, R. Schmid, T. Sierra, M.J. Quejido, A.J. Sánchez, D.M. Cardona, A.I. Fernández, M. and Vera, R. 2006. Mercury content in vegetation and soils of the Almadén mining area (Spain). *Science of The Total Environment* 368: 79-87.
- Rahimi, E., Hajisalehi, M., Kazemeini, H.R., Chakeri, A., Khodabakhsh, A., Derakhshesh, M., Mirdamadi, M., Ebadi, A.G., Rezvani, S.A. and Kashkahi, M.F. 2010. Analysis and determination of mercury, cadmium and lead in canned tuna fish marketed in Iran. *African Journal of Biotechnology* 9(31): 4938-4941.
- Rohman, A., Riyanto, S., Yuniarti, N., Saputra, W. R., Utami, R. and Mulatsih, W. 2010. Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus Lam*). *International Food Research Journal* 17: 97-106.
- Rohman, A. Sugeng, R. dan Hidayati, N.K. 2007. Aktivitas antioksidan, kandungan fenolik total, dan flavonoid total daun Mengkudu (*Morinda citrifolia L.*), *Agritech* 27(4): 147 – 151.
- Taverniers, I. De Loose, M., and Van Bockstaele, E. 2004. Trends in quality in the analytical laboratory: Analytical method validation and quality assurance. *Trends in Analytical Chemistry* 23: 535 – 552.
- Tong, S., von Schirnding, Y.E., Prapamontol, T. 2000. Environmental lead exposure: a public problem of global dimension. *Bulletin of the World Health Organization* 78: 1068–1077.
- Tsai, T.H., Tsai, P.J. and Ho, S.C. 2005. Antioxidant and anti-inflammatory activities of several commonly used spices. *Journal of Food Science* 70(1): C93 – C97.
- Voegborlo, R.B. and Akagi, H. 2007. Determination of mercury in fish by cold vapour atomic absorption spectrometry using an automatic mercury analyzer. *Food Chemistry* 100: 853–858
- Wagner, G.J. 1993. Accumulation of cadmium in crop plants and its consequences to human health. *Advances in Agronomy* 51: 173–212.