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Research Paper

Validation of Mercury Speciation Analysis in River Around Artisanal Small-Scale Gold Mining Area in West Nusa Tenggara, Indonesia

Dhony Hermanto[1]*, Nurul Ismillayli[1], Nindi Herdiyanti[1], Siti Raudhatul Kamali[1,2], Soraya Aulia[3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram, 83125, Indonesia*
[2]*Department of Applied Chemistry, Chaoyang University of Technology, Wufeng, 41349, Republic of China*
[3]*Laboratory Center of Health Testing and Calibration, West Nusa Tenggara Provincial Health Office, Mataram, 83121, Indonesia*
***Corresponding author**: dhony.hermanto@unram.ac.id

Abstract
A method for determining mercury concentration using a mercury analyzer in a river water sample was validated according to ISO/IEC 17025. Analytical performance including linear range, limit of detection, precision and accuracy were evaluated. Mercury speciation profile was obtained from Pelangan River at three areas within Dusun Rambut Petung, an area with the highest amount of artisanal small-scale gold mining (ASGM) in Lombok, West Nusa Tenggara. Then, their concentration in each species was measured using sequential extraction. Good curve linearity was obtained in the concentration range of 0.1-5.0 µg/L and the limit of detection was 0.014 µg/L. The developed method has good precision and accuracy with a RSD value <10% and a recovery of 94.16-101.91%. The detected fraction of mercury in the Pelangan river is organomercury, elemental mercury, and sulfide-bound species with each concentration of 0.732±0.032; 0.350±0.027; and 0.850±0.027 µg/L, respectively. The measurement results showed conformity with the reference method using CV-AAS. Therefore, this method can be applied to determine mercury levels in water for monitoring environmental quality.

Keywords
Validation, Mercury Analyzer, Mercury Speciation, the Pelangan River, Rambut Petung District

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1. INTRODUCTION

Gold as a mineral resource with a very high economic value is available in plentiful supply in Sekotong District, Lombok Island, Indonesia. It has led to rampant mining activities, especially ASGM which began in mid-2008 until now (Krisnayanti et al., 2016). ASGM is carried out by people in the Sekotong area using the amalgamation method, which is a traditional method of separating gold from the rock using mercury, a very toxic and dangerous chemical (Brooks et al., 2017; Spiegel et al., 2018). It is estimated that between 410 and 1400 tonnes of mercury are emitted worldwide through ASGM each year, equivalent to 37% of global mercury emissions from anthropogenic source (Esdaile and Chalker, 2018; Seccatore et al., 2014). Hence, monitoring mercury from gold mining waste is an important issue that needs serious handling.

Mercury is a pollutant that has received the most attention because of its high toxicity and persistence and accumulative behavior in the environment. Considering that mercury is volatile in metalic Hg^0 form, can transport through the atmosphere at considerable distances. Mercury also exist as organo-Hg that can bioaccumulate in organisms (UNEP, 2013). Mercury in the environment exists in different molecular forms according to specific biogeochemical transformations and ecotoxicity. Inorganic ionic mercury (Hg^{2+}) is a common form/species found in water samples (Hermanto et al., 2019; Hermanto et al., 2022). Particular attention is given to mercury which undergoes a natural transformation called organomercury compounds (Hrubaru et al., 2018), including the in-situ formation of methylmercury (MeHg) and dimethylmercury (Me_2Hg). Although it usually represents a small portion of total mercury (Hg_{TOT}) in aquatic environments, methylmercury is highly toxic due to its tendency to bioaccumulate and biomagnify in the aquatic food chains (Živković et al., 2017; Omwoma et al., 2017). Determination of Hg_{TOT} concentration is not sufficient to understand its presence in the environment (Spyropoulou et al., 2018), each species has different mobility and affect its availability, as well as its toxicity. According to The International Union of Pure and Applied Chemistry (IUPAC), speciation analysis is an analytical activity for the identification and measurement of one or several individual chemical forms of an element including mercury (Stoichev et al., 2006;

Templeton et al., 2000).

Validation of analytical methods as a crucial step of quality assurance is needed to obtain reliable results in quantitative analysis (Eka et al., 2012). Based on ISO/IEC (2017), the validation of the analytical method intends to assure that the method meets the acceptance criteria. Reference method for the determination of mercury in the water sample by cold vapor-atomic absorption spectrometry (CV-AAS), is recommended by (EPA, 1994; Kallithrakas-Kontos and Foteinis, 2016). Until now, the application of a mercury analyzer for the species mercury quantification in aquatic samples has not been reported. The Pelangan River is one of the rivers that flow in Sekotong used for irrigation and it passes Dusun Rambut Petung. Aquatic samples were obtained from the Pelangan River in Dusun Rambut Petung Sekotong-Lombok. This area was chosen because it is the most plentiful area of ASGM, with more than 900 ball mills for crushing gold ores. Hence, it is essential to assure its quality control including the level of mercury. Developing and validating a mercury analyzer for the quantification of species mercury in Pelangan River, Dusun Rambut Petung Sekotong-Lombok Island, was conducted in this study.

2. EXPERIMENTAL SECTION

2.1 Materials and Instrument

The sample was Pelangan river water that was collected based on the sampling technique criteria. Mercury standard solution (1000 mg/L) was purchased from Sigma Aldrich (Steinheim, Switzerland). The rest of the reagent was obtained from Merck (Darmstadt, Germany) with a pro analyst grade classification. Distilled and deionized water were used as solvents. All glassware used in this study was soaked in a detergent solution, then rinsed with distilled water.

Mercury Instrument® Analytik Jena Mercur Duo and Mercur Duo Plus (Jena, Germany) based on atomic absorption without enrichment was used for mercury determination. The instrument was operated via WinAAS for Mercur. The instrument was equipped with Hg low-pressure lamp UVU5 with beaker electrode, detector Photomultiplier (PMT) 1P28 with 9-stage. The measurement wavelength was 253.7 nm, the airflow was set at 10 L/h and the sensitivity of the analytical balance was 0.1 mg.

2.2 Sample Station

The study was located in the Pelangan river, Dusun Rambut Petung, in Sekotong district, Lombok Island in the latitude between 8°48'37" to 8°49'4"S and longitude between 115°56'40" to 115°57'36"E. The river that crosses this village has a length of about 2.2 km and a width of 3.5 m with an average depth of the river is 1.5 m. Water samples were collected from three sampling stations. The stations are selected depending on the estimated water quality and pollution levels when observing the study area. Station 1 was in the direction of the upstream river, station 2 was in the mining waste disposal location and station 3 is in the downstream direction. Water samples were collected from 3 sampling stations during the period in April 2021 (in the rainy season with a river flow speed of 10 ms^{-1}).

2.3 Sample Collection

For sampling, 500 mL of water sample was placed in a plastic bottle with a double plug from each sampling station. Before taking samples, the bottles were cleaned and washed with a detergent solution then rinsed with 5% HNO_3 and left overnight. The bottles were finally rinsed with deionized water and dried. At each sampling station, sample bottles were rinsed at least three times before sampling. The prepared sample bottles were immersed about 10 cm below the surface of the water. The sample was acidified with 10% HNO_3, then the bottles were carefully sealed, marked with their respective identification numbers, placed in an ice bath, and taken to the laboratory. Samples were filtered through a 0.45 μm micropore membrane filter and frozen at 4°C for preservation and avoiding further contamination until the analysis process was carried out.

2.4 River Water Analysis

The standard curve for mercury solution was made in various mercury concentration, namely 0.1; 0.5; 1.0; 2.5 and 5.0 μg/L. A blank solution was prepared by mixing the standard reagent for 100 mL of sample (consisting of 1000 μL KBr-$KBrO_3$ and 50 μL hydroxylamine hydrochloride 12%) and dd water up to a volume of 100 mL. Variation of mercury concentration was obtained by mixing some mercury stock solutions with standard reagent and diluted with dd water according to the required concentration. Determination of the total mercury concentration in river water samples was carried out according to the research method conducted by Gill and Bruland (1990), which was obtained by mixing 50 mL of the sample with 10 mL of 4% $NaBH_4$ solution (w/v) in 0.15 N NaOH, then adding a standard reagent solution. All solutions were measured for absorbance with a mercury analyzer at a wavelength of 253.7 nm and the repetition was carried out 3 times.

2.5 Validation Method

The validation method was carried out by assessing several analytical numbers based on the international conventions on the analytical method (Magnusson, 2014; ICH, 1994), such as linearity and dynamic range, sensitivity expressed by detection limits (LOD), and quantitation limits (LOQ), precision, accuracy and performance tests compared to reference methods recommended by EPA methods 245.1 for the determination of mercury in the water sample, namely CV-AAS (EPA, 1994; Kallithrakas-Kontos and Foteinis, 2016). The performance test was carried out for the determination of Hg_{TOT} in aquatic samples.

2.6 Mercury Speciation

Determination of the concentration of Hg metal speciation using a stepwise extraction method was conducted based on the research method by Boszke et al. (2008). Before the measurement, standard reagent and solution were added to each

fraction and the absorbance of each fraction was measured using a mercury analyzer at a wavelength of 253.7 nm.

Fraction 1 was the organomercury fraction that was obtained by adding 50 mL of river water sample with 30 mL $CHCl_3$ and shaking for 3 min. Furthermore, the sample mixture was centrifuged at 3000 rpm for 15 min and the results were decanted. The organic phase was extracted again using 10 mL of 0.01 M $Na_2S_2O_3$ for 3 min, then it was separated. The upper phase (sodium thiosulfate) was added with the measuring agent. Fraction 2 was water-soluble. The water phase from Fraction 1 was filtered with a filter membrane then put in a test tube and added with the measuring agent. Fraction 3 was acid-soluble. It was obtained by adding 50 mL of river water sample with 25 mL of 0.5 M HCl and centrifuging at 3000 rpm for 15 min. The result was filtered with a filter membrane then put into a test tube and the measuring agent was added. Fraction 4 is the associated fraction in humic material, this was obtained by adding 50 mL of river water sample with 30 mL of 0.2 M NaOH and centrifuging at 3000 rpm for 15 min. The result was filtered with a filter membrane then put into a test tube and the measuring agent was added. Fraction 5 was an elemental mercury fraction that was obtained by digesting 50 mL of river water sample with 12 mL of 37% HCl and 4 mL of 65% HNO_3. The mixture was put into a test tube and the measuring agent was added. Fraction 6 was the residual fraction, it was obtained by heating 50 mL of river water at 150°C for 30 min and digested using 12 mL of 37% HCl and 4 mL of 65% HNO_3. The mixture was put into a test tube and the measuring agent was added.

3. RESULTS AND DISCUSSION

To evaluate the validity of the proposed method, the analytical performance characteristics for the determination of mercury in aqueous samples were estimated. Some of the analytical performance characteristics were determined by a calibration curve, as shown in Figure 1. The first evaluation of the analytical performance characteristic is the linear range. The linearity of the response was studied using a calibration curve for a standard solution of mercury by plotting the absorbance against the mercury concentration.

Figure 1 shows a plot of the calibration curve between mercury concentration and absorbance measured at a wavelength of 253.7 nm. Good curve linearity is obtained, with a correlation coefficient, R^2=0.9998(r»1) in the mercury concentration range between 0.0 to 5.0 μg/L. According to Magnusson (2014), the analytical characteristic is linear over a given concentration range if R^2 obtained is higher than 0.995.

The LOD is the lowest detectable concentration of the analyte and is reliably distinguished from zero concentration, it should not be measured. While, the LOQ is the lowest concentration of analyte that can be quantized with an acceptable level of precision (González and Herrador, 2007). In determining LOD and LOQ, the sample blank solution was measured. LOD and LOQ were calculated as 3×3STDEV/b and 10STDEV/b, respectively, where STDEV is the standard

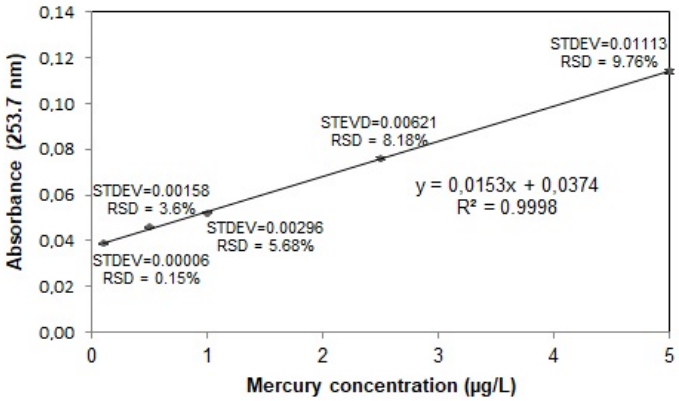

Figure 1. The Calibration Plot of Hg Masurement

deviation of the analyte response and b is the slope of the calibration curve (Figure 1) (Magnusson, 2014). In this proposed method, the LOD and LOQ are 0.014 g/L and 0.016 g/L, respectively, found to be more sensitive than conventional atomic absorption spectroscopy for the determination of mercury with an LOD of 0.12 g/L (Hartwig et al., 2019).

In this study, precision was measured as the relative standard deviation (RSD) of mercury concentration. The accuracy of the mercury analysis shows that the response of the mercury standard solution is always reproducible, including errors due to the operating system, but not errors due to handling and sample preparation (Ertas and Tezel, 2004). To assess the accuracy of the analytical method, measurements were made in a repeatable condition. In Figure 1, it can be seen that the RSD of mercury analysis is less than 10%. The maximum acceptable RSD value is 32% (for analyte concentration <10.0 μg/L), hence the precision of the proposed method is excellent (González and Herrador, 2007).

Table 1. Accuracy Studies Data for Mercury Analyzer

Spiked analyte concentration (μg/L)	Found analyte concentration (μg/L)	STDEV	RSD (%)	Recovery (%)
2.0	2.01	0.03	1.49	95.62
3.0	2.83	0.05	1.77	94.16
4.0	3.95	0.03	0.76	101.9

Recovery study was carried out to confirm deficiency or loss of analytes or contamination during sample preparations and matrix disturbances during measurement. This parameter is used to evaluate the accuracy of the analytical method (Ertas and Tezel, 2004). Recovery is determined by the spiking technique, the concentration of the known mercury solution is added to the sample, then the resulting spike is measured, calculated, and compared with the added mercury solution (concentration is known). All analytical steps were carried out in three replications with three different levels of mercury concentration. The recovery values are in the range 94.16-101.91%

(Table 1), is acceptable because it is in the range of 60-115% (for analyte concentration <10.0 μg/L) (Taverniers et al., 2004). Hence, the method developed is accurate for calculating mercury samples in the aqueous system.

The performance of the mercury analyzer was evaluated in the analysis of aquatic samples. The standard addition method was used by spiking different amounts of mercury into the sample. The results of the determination are summarized in Table 2. Statistical analysis using ANOVA showed that each station has real different results, $F_{stat} > F_{crit}$ (F_{stat}=41.421, F_{crit}=9.552), P_{value}<0.05 (0.006), means that H_0 is rejected. While, statistical analysis for mercury analyser and CV-AAS comparison obtained that $F_{stat} < F_{crit}$ (F_{stat}=0.087, F_{crit}=7.708), P_{value}>0.05, means that H_0 is accepted (there is no significant different between mercury analyzer and CV-AAS measurement results). The results indicated that the developed mercury analyzer has very good characteristics for the determination of mercury in the aquatic sample. The results shown in Table 2 were based on the reference method using CV-AAS.

Determination of the concentration of various types of mercury species and their availability in river water (in station 2, mining waste disposal site) can be done simply by sequential extraction. It can provide detailed information about the origin, stages of events, possible biological and physicochemical preparations, movement and displacement of metals as well as partition metal particulates in the environment (Boszke et al., 2008). In this study, there were six stages of sequential extraction to determine the various fractions of mercury in various types of speciation including the organomercury fraction, the water-soluble fraction, the acid-soluble fraction, the mercury fraction bound to the humus material, the elemental mercury fraction, and the mercury fraction bound to sulfides. Result for determination of mercury speciation in the Pelangan river, Rambut Petung, Sekotong, Lombok Island as shown in Figure 2.

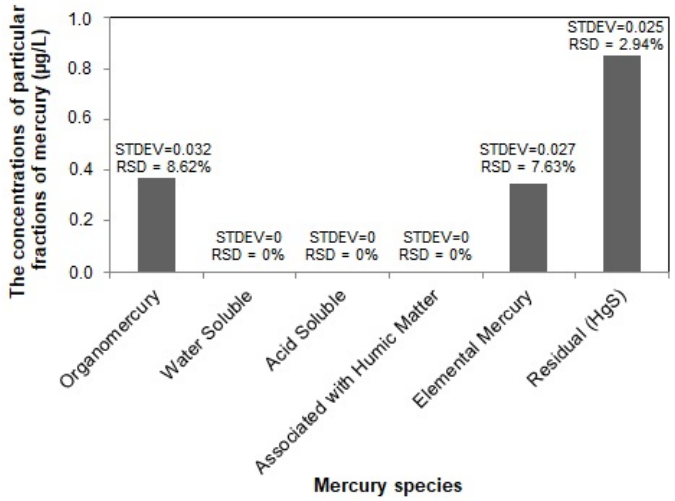

Figure 2. Mercury Speciation in the Pelangan River, Rambut Petung Sekotong

In the organomercury fraction, mercury compounds are bonded directly to the carbon atoms of organic matter (e.g. CH_3Hg). This species has a higher toxicity than inorganic mercury species and is the most easily displaced so that it is easier to accumulate in living things than other heavy metals. Its ability in forming strong binding with sulfhydryl proteins, encourage this fraction accumulated in the tissue of a living organism. In the extraction stage, chloroform, an organic solvent, and nonpolar were used to extract the organomercury fraction. The selective method of organomercury extraction was the pre-concentration process, where the obtained extract in organic solvents was extracted again using sodium thiosulfate to obtain the concentration of this species (Boszke et al., 2008). Methylmercury, methyl group bonded to mercury(II), is one of the organomercury may undergo complexation reaction with thiosulphate ion (Lu et al., 2014), according to the chemical reaction Equation 1.

$$H_3C - Hg^+ + 2(S_2O_3)^{2-} \rightarrow Hg(S_2O_3)_2{}^{2-} + -CH_3 \quad (1)$$

Sodium thiosulfate is an excellent chelating ligand to bind mercury, where thiosulfate has a sulfide group that has a tends to bind strongly to mercury. The contribution of organomercury species to the total mercury concentration in this study showed a level of 0.732±0.032 μg/L, as shown in Figure 2. The contribution value of this species was the second-highest in mercury speciation compared to other fractions. Due to its toxicity and bioaccumulation in living things, serious attention is needed on this issue.

The water-soluble fraction of mercury is a species that is easily moved due to its solubility in water. Usually, mercury is not a water-soluble ionic species but it is a species bound to organic matter (without carbon-Hg bonds) or suspended mineral particles. The contribution of water-soluble mercury species to the total concentration of mercury in this study is not detected, as shown in Figure 2. The undetectable mercury metal was probably caused by slow river flow that cause a methylation process, in which mercury(II) methylation process was caused by sulfate-reducing bacteria under anoxic conditions. In addition, mercury(II) could be bound to hydroxides, chlorides, and sulfides found in river water, then coagulated and was precipitated. The contribution value of this species showed that it has high mobility so that it is not detected in the water phase (Balogh et al., 2008).

Acid-soluble fraction is defined as mercury species released under acidic conditions and sensitive to pH changes of river water. Commonly, mercury is bound to iron monosulfide, iron and manganese hydroxide, and carbonate. These compounds can include species that are bound to organic matter and adsorbed on mineral surfaces (Boszke et al., 2008). As shown in Figure 2, the contribution of this mercury species in this study is not detected, which indicated that this species was not found in the dissolved water phase or its concentration is lower than LOD (0.014 μg/L).

Table 2. Determination of Mercury in Aquatic Samples Using Mercury Analyzer and CV-AAS

Sample	Found Mercury concentration (μg/L) by mercury analyzer	by CV-AAS	Relative Error (%)
Station 1 (8°48'37"S,115°56'40E)	0.95	0.83	0.126
Station 2 (8°48'50"S,115°56'48E)	1.571	1.452	0.082
Station 3 (8°49'4"S,115°57'36E)	1.28	1.29	0.008

Notes: average triplicate of measurements

The fraction associated with humus material is in the form of organic material/humus substances such as humin, humus acid, and fulvic acid which are important components of sediment and soil. Humic acid does not dissolve at acidic pH but dissolves at alkaline pH conditions, fulvic acid dissolves in water at all pH conditions, while humin does not dissolve under acidic or alkaline conditions (Sparks, 2003). Under certain conditions, the humus substance plays a role in metal bonds. Organic mercury is mercury in the form of a complex mercury(II) with ligands such as humus, fulvic acid, amino acids (without Hg-carbon bonds). Mercury is bound to organic material with the thiol (R-SH), disulfide (R-SSR), or disulfane (R-SSH) functional groups (Boszke et al., 2008). On the water surface, the humic content is expressed as dissolved organic carbon (DOC). Humic acid does not dissolve at pH<2 but it dissolves in alkaline conditions so that in this fraction an alkaline process is carried out by adding NaOH to determine the Hg bound to humic acid. In general, the organic ligands in humus material in the form of humic acid and fulvic acid are slightly acidic because the metal easily binds to the acidic humus material. The contribution of mercury species bound to humus material to the total concentration of mercury in this study is not detected (LOD of 0.014 μg/L), as shown in Figure 2. It was possible that the humus material bind to other organic matter or clay then settles on the bottom of the river so that it was not found in the sample.

In the elemental fraction, mercury is a species of mercury in the form of pure metal mercury (Hg) which is easily volatile. The use of aqua regia (a mixture of HNO_3 and HCl) as a strong oxidizer causes mercury to be oxidized to Hg^{2+} according to the reaction Equation 2. The contribution of elemental mercury species to the total mercury concentration in this study showed levels of 0.350±0.027 μg/L, as shown in Figure 2.

$$Hg + HNO_3 + 3HCL \rightarrow Hg^{2+} + Cl_2 + NOCl + 2H_2O \quad (2)$$

The fraction bound to the sulfide is a non-mobile fraction. In this fraction, the sample was digested by heating to remove disturbing substances from other organic materials. Then it is oxidized using aqua regia to obtain $HgCl_2$ (Mikac et al., 2002) according to Equations 3 and 4.

$$HNO_3 + 3HCl \rightarrow NOCl + Cl_2 + 2H_2O \quad (3)$$

$$HgS + Cl_2 \rightarrow HgCl_2 + S \quad (4)$$

The contribution of sulfide-bound species to the total concentration of mercury in this study showed levels of 0.850±0.027 μg/L, as shown in Figure 2. The nature of mercury sulfide which is stable and difficult to dissolve in water also allows the greatest concentration value obtained compared to metal mercury and organomercury. The total mercury concentration calculated as the sum of the mercury concentration in individual fractions by stepwise extraction obtained in this study was 1.57±0.014 μg/L.

4. CONCLUSION

A method validation on mercury analyzer for mercury determination followed by sequential extraction for mercury speciation was conducted in Pelangan River, Rambut Petung District. The result showed low LOD of 0.014 μg/L, good linear range concentration of 0.0-5.0 μg/L, good precision, and accuracy with RSD <10%, and recovery of 94.6-101.91%. There were three forms of mercury detected, namely organomercury of 0.732±0.032 μg/L, elemental mercury of 0.350±0.027 μg/L, and sulfide-bound mercury of 0.850±0.027 μg/L. The agreement of measurement result between the developed method and CV-AAS as a reference method indicated that this method can be used for mercury determination.

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