

Total arsenic in tuna fish candidate reference material preparations: Homogeneity and stability testing

T A Koesmawati^{1*}, Buchari² and J Ng³

¹ Research Unit for Clean Technology, Indonesian Institute of Sciences (LIPI), Komplek LIPI Bandung, Jalan Sangkuriang, Gedung 50, Bandung, 40135, Indonesia

² Analytical Chemistry Department, Bandung Institut of Technology (ITB), Jalan Ganeca 10, Bandung 40135, Indonesia

³ The University of Queensland, National Research Centre for Environmental Toxicology (Entox), Brisbane 4108, Queensland, Australia

*E-mail: tiny.agustini@gmail.com

Abstract. Reference material plays an important role in analytical measurements. Total arsenic was analyzed in tuna fish samples using three different preparation methods for stability. The stability of total arsenic in tuna fish was evaluated over 12 months, under room temperature storage condition. Each measurement was conducted in triplicate in three different laboratories. Statistical analysis was carried out using Student's t-test. Two different methods were conducted to confirm homogeneity of the candidate reference material in this study. Random bottle number was applied in this analysis. Statistical analysis was carried out and the homogeneity for total arsenic was calculated using the one-way analysis of variance (ANOVA). The statistical results showed no significant changes in stability and the sample was homogeneous. The candidate reference material developed in this study demonstrated its suitability for quality assurance of the total arsenic measurement in tuna fish.

1. Introduction

Arsenic is a metalloid widely distributed in the earth's crust and present at an average concentration of 2 µg/g. Arsenic can exist in four valences states: -3, 0, +3 and +5. Under reducing conditions, arsenite (As (III)) is the dominant form; arsenate (As (V)) is generally the stable form in oxygenated environments. Arsenic causes adverse health effects including cancers in human. At present, millions of people worldwide suffer from chronic arsenic poisoning [1,2] mainly due to consumption of arsenic-contaminated water and food. Long-term exposure to arsenic in drinking-water is causally related to increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been reported to be associated with ingestion of drinking-water at concentrations 50 µg arsenic/L. Occupational exposure to arsenic, primarily by inhalation, is causally associated with lung cancer. Exposure-response relationships and high risks have been observed. Increased risks have been observed at cumulative exposure levels $\geq 0.75 \text{ (mg/m}^3\text{)} \times \text{year}$ (e.g. 15 years of exposure to a workroom air concentration of 50 µg/m³).

Various of instrumental techniques for arsenic determination is available such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma atomic



emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and voltammetry. ICP-MS as an example, can serve as element-specific detectors when coupled to chromatographic separation techniques such as HPLC and GC. The use of CRM on testing and calibration process is a requirement from ISO/IEC 17025-2005. This material is an essential tool to the quality control of measurement methods [3]. The certification of a reference material is carried out according to the requirements of ISO Guide 34 series [4]. The ISO Guide 35 states that the certification process of a CRM requires a careful study of all sources of uncertainty that can cause impact on the validity of the certified values. In general, these sources are relevant to characterization uncertainty, homogeneity uncertainty, uncertainty stability inherent to transport, uncertainty stability inherent to the storage, that are essential for the development and certification of a CRM [5]. The homogeneity study is necessary in the certification process of a lot of a RM to demonstrate that the units of this lot are sufficiently homogeneous amongst themselves. The homogeneity should be evaluated between different units of the candidate CRM lot (bottles at this case) and also within the same unit.

Total arsenic in tuna fish is usually found in relative very low concentrations of below 10 µg/g. Two major difficulties in the measurement of total arsenic in tuna fish are the concentration and its matrix interferences [6–9]. Accuracy and precision in its measurement is mandatory for an accredited testing laboratory, therefore the availability of a suitable reference material (RM) is necessary [10,11]. RM is a material or substances with one or more of its properties being sufficiently homogeneous and stable that are well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials (ISO Guide 30-Ref C1). RM is necessary in method development and validation, estimation of measurement uncertainty, internal quality control, proficiency testing, and training. Both homogeneity and stability are essential in the preparation of an RM of biological origin. The homogeneity test is one of the most important steps in the development and production of a CRM [12,13]. In homogeneity studies, the various units of a material batch are important for establishing the properties of the batch. To evaluate the units, the choice of the unit should be performed randomly for that assessment to be representative of the total quantity. The National Research Council Canada (NRC) developed certified reference material (CRM) DORM-2, dogfish muscle CRM for trace metals, which was replaced by CRM DORM-3, fish protein CRM for trace metals. However, in Indonesia CRM is difficult to purchase. The objective of this study was to provide an RM which can be used as in-house reference material for quality control for the determination of total arsenic in tuna fish samples for Indonesian testing laboratories.

2. Experimental

2.1. Preparation of a reference material

Tuna fish samples were collected from Jakarta Bay. The tuna meat was minced, homogenized, and freeze-dried for 24 hr. Liquid nitrogen was added to the dried samples and grinded using an agate mortar. The powdered samples were sieved (100 mesh) then packed in dark glass bottles of 1 g lots.

2.2. Tuna fish analysis

The 50 mg of dried sample was accurately weighed. Three different methods of preparation were conducted, namely microwave assisted acid digestion, dry ashing, and waterbath acid digestion. The digest was diluted 5 times before being measured by ICP-MS. Measurements were validated using CRM DORM-2 and DORM-3.

2.3. Homogeneity test

Total arsenic was assessed using the different methods in two different laboratories. Twenty of 1 g lots were prepared for the homogeneity test. Random bottle number was applied in this analysis. Statistical analysis was carried out and the uncertainty of homogeneity for total arsenic was calculated using the one-way analysis of variance (ANOVA). In homogeneity testing, two importance type of methods should be noticed. Firstly, the within-bottle homogeneity, which dictates the minimum sample intake,

for which the established uncertainty is still valid. Secondly, the between-bottle homogeneity, which deals with the bottle-to-bottle or unit-to-unit variation. Separation between the heterogeneity and the variability of measurement should be established. Therefore, the within-bottle homogeneity study was carried out in a very small amount of sample; so that the between-portions effect can be quantified. When the test was completed, the minimum sample intake was calculated. On the other hand, a between-bottle homogeneity test was conducted with the optimum sample intake to minimize analytical variation. This intake should be higher than the minimum sample intake described above. When between-unit variation was able to quantify, the two effects could be separated by analysis of variance (ANOVA) [14,15].

2.4. Stability test

Two types of stability test were performed. Firstly, study at elevated temperature to elucidate whether any degradation occurred during transport. Short duration stability was carried out for not longer than 4 weeks. Secondly, study was performed at storage temperature to obtain information about the stability during storage. Usually, a stability study consists of a series of measurements performed at different times. Previous study was conducted in storage temperatures at 18 and 60°C; the reference temperature was 70°C. The storage times were 0, 1, 2, and 4 weeks. After the indicated storage periods, samples were transferred to storage at 70°C until analysis [16]. Analytical variation can be reduced by using either a method with less variation or by the isochronous measurement scheme [17], in which repeatability rather than reproducibility conditions are applied. The influence of analytical variation can be reduced further by performing higher numbers of replicates per time and by increasing the length of the stability study. In this study, the stability of total arsenic in tuna fish was evaluated over 12 months, under room temperature storage condition. Each measurement was conducted in triplicate in three different laboratories and statistical analysis was carried out using Student's t-test.

3. Results and Discussion

In the process of RM preparation, homogeneity is the first consideration. The homogeneity of a liquid or gases type of RM can be easily obtained by repetitive shaking. However, it is more difficult to homogenize a solid type of RM. Two different methods were conducted to confirm homogeneity of the candidate RM in this study.

Table 1. Results of the homogeneity test for total arsenic in the candidate reference material.

Bottle no.	ICPMS ^a	Bottle no.	ICPMS ^b
	Conc (µg/g)		Conc. (µg/g)
2	3.36	15	3.44
23	3.50	21	3.64
11	3.42	12	3.53
14	3.58	22	3.36
13	3.86	8	3.34
3	3.93	29	3.34
17	3.61	25	3.37
F_{calc}	3.71		
F_{crit}	4.75		

ICPMS^a : Institut für chemie, Karl Franzens University, Graz, Austria
(microwave assisted acid digestion)

ICPMS^b : The University of Queensland, National Research Centre for
Environmental Toxicology, Brisbane, Australia (water bath acid digestion)

Table 2. Statistical calculation ANOVA single factor.

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Column 1	7	25.26	3.608571	0.046081		
Column 2	7	24.02	3.431429	0.013148		

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F_{crit}</i>
Between Groups	0.109829	1	0.109829	3.708635	0.078152	4.747225
Within Groups	0.355371	12	0.029614			
Total	0.4652	13				

Another method of homogeneity testing was conducted based on ILAC Guide [14,15] using equation as follows:

$$MSB = \frac{\sum [(a_i + b_i) - \bar{X}_{(a_i + b_i)}]^2}{2(n-1)} \quad (1)$$

$$MSW = \frac{\sum [(a_i + b_i) - \bar{X}_{(a_i + b_i)}]^2}{2n} \quad (2)$$

$$Sd_{\text{sampling}} = \sqrt{(MSB - MSW) / 2} \quad (3)$$

MSB = mean square between; MSW = mean square within; Sd = standard deviation

The homogeneity was measured in four criteria:

- First criteria, is the value of $MSW/MSB < F_{\text{critical}}$
- Second criteria, is the value of standard deviation sampling $< 0.3 \sigma$
- Third criteria, is the value of standard deviation sampling $< 0.3 \times \text{CV Horwitz prediction}$
- Fourth criteria, is the value of standard deviation sampling $< \text{CV Horwitz prediction}$

From the data above, the MSB and MSW value were found to be 0.0140 and 0.0388, respectively. The F statistic value was calculated from $MSW/MSB = 2.77$, this value was lower than F critical from F table (at $\alpha = 0.05$) of 3.71. In this study, the RM was homogeny.

Table 3. Results of stability test of total arsenic in the candidate reference material.

	Total arsenic concentration ($\mu\text{g/g}$)						
	Dec	Feb	April	June	August	Oct	Dec
	3.53	3.18	3.77	3.47	3.44	3.44	3.44
	3.31	3.54	3.69	3.78	3.12	3.41	3.64
	3.44	3.12	3.89	3.71	3.46	3.39	3.53
Average	3.43	3.28	3.78	3.65	3.34	3.41	3.54
Standard deviation	0.11	0.23	0.10	0.16	0.19	0.03	0.10
t_{calc}	2.13	1.32	0.32	1.87	1.52	0.54	

Beside homogeneity, the stability of the RM is important. In chemical testing laboratories, dried samples are usually stored at room temperature. Therefore, the stability test for this candidate RM was performed over 12 months at room temperature and measured in three different laboratories. The statistical results showed no significant changes in stability as measured by the total arsenic concentration, because t_{calc} were lower than t_{crit} for the degree of freedom of $n-2$ and confidence level of 95%, as shown in Table 3.

The concentration of total arsenic was found steady for 12 months as shown in Figure 1, with the linear equation of $y = 0.0027x + 3.4739$.

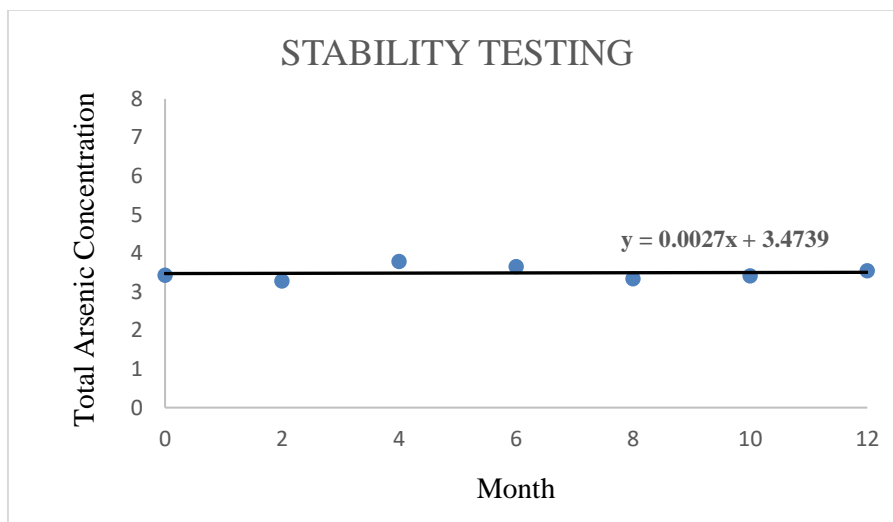


Figure 1. Total arsenic concentration in 12 months storage.

CRM DORM-2 and DORM-3 were periodically analyzed for more than nine years. It was found physically and chemically stable over that time period [18,19].

4. Conclusions

No significant difference was observed among aliquots of the candidate RM. It also showed that there was no significant changes in stability of the candidate material over 12 months at room temperature. The candidate RM developed in the present study is suitable for quality assurance for the determination of total arsenic in tuna fish as well as satisfactory for its homogeneity and stability. The candidate RM also demonstrated its suitability for quality assurance of the total arsenic measurement in tuna fish.

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