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Comparison of Microwave Assisted Acid Digestion Methods for ICP-MS Determination of Total Arsenic in Fish Tissue

(Perbandingan Kaedah Penghadaman Asid dengan Bantuan Mikrogelombang bagi Penentuan Jumlah Arsenik dalam Tisu Ikan Menggunakan ICP-MS)

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ABSTRACT

Fish is one of the most important sources of arsenic exposure in human diet and the Food Safety and Quality Division, Ministry of Health since 2007 has required routine monitoring of total arsenic in seafoods such as fish. This study describes an improved extraction method of total arsenic in fish using microwave assisted acid digestion procedure before being analysed by inductively coupled plasma mass spectrometry (ICP-MS). The parameters studied were pre-treatment of sample, digestion temperature, time programme and the chemicals (HNO_3/H_2O_2) used. Arsenic contents in fish samples under these conditions were compared using the standards additions technique. Microwave assisted acid digestion method with a combination of ultrapure concentrated nitric acid (HNO_3) to concentrated hydrogen peroxide (H_2O_2) at a ratio of 7 mL: 1 mL, run time of 25 min and digestion temperature of 200°C with no pre-treatment was found to have recovery of 100.7% as compared with other digestion procedure where the recovery were 115.5, 111.6 and 101.8%. Validation using certified reference material (CRM) of fish tissue (DORM-3) showed a recovery of 101.4 ± 2.5% for total arsenic from the CRM.

Keywords: Arsenic; certified reference material; fish; inductively coupled plasma mass spectrometry; microwave assisted acid digestion

ABSTRAK

Ikan adalah salah satu daripada sumber yang paling penting bagi pendedahan arsenik dalam diet manusia dan sejak 2007 Bahagian Keselamatan dan Kualiti Makanan, Kementerian Kesihatan telah menjalankan pemantauan rutin bagi jumlah arsenik dalam makanan laut seperti ikan. Kajian ini menerangkan kaedah pengekstrakan yang diperbaiki bagi jumlah arsenik dalam ikan dengan menggunakan prosedur penghadaman mikrogelombang dengan bantuan asid sebelum dianalisis dengan menggunakan spektrometri jisim plasma gandingan aruhan (ICP-MS). Parameter yang dikaji adalah pra-perlakuan sampel, suhu penghadaman, program masa dan bahan kimia (HNO_3/H_2O_2). Kandungan jumlah arsenik dalam sampel ikan dalam keadaan tersebut dibandingkan dengan menentukan perolehan penambahan bahan piawai jumlah arsenik. Kaedah mikrogelombang dengan bantuan asid dengan gabungan asid nitrik (HNO_3) serta hidrogen peroksida pekat (H_2O_2) tulen pada nisbah 7 mL:1 mL dengan masa analisis selama 25 min dan suhu penghadaman 200°C serta tiada pra-perlakuan sampel, didapati mempunyai nilai perolehan 100.7% berbandingkan dengan kaedah lain dengan nilai perolehannya adalah 115.5, 111.6 dan 101.8%. Validasi telah dijalankan dengan menggunakan bahan rujukan yang disahkan atau (CRM) tisu ikan (DORM-3) menunjukkan perolehan 101.4 ± 2.5% bagi jumlah arsenik dari CRM.

Kata kunci: Arsenik; bahan rujukan yang disahkan; ikan; mikrogelombang dengan bantuan asid; spektrometri jisim - plasma gandingan aruhan

INTRODUCTION

Arsenic and its compounds are identified as Group 1 carcinogens to humans by the International Agency for Research on Cancer (IARC 1994). Arsenic can be a major source of DNA disruption and is known to cause skin, lungs, liver and spleen cancer (FSA 2005; Hung et al. 2004). This is because sulphydryl groups are frequently found in DNA-binding proteins and arsenic has the tendency to create bond with suphyryl, thus disrupt the DNA mechanism (FSA 2005).

Arsenic exists in the environment from natural sources such as volcanic activities and through weathering (FSA 2005; Hymer & Caruso 2004; Reilly 1980). Excessive usage of agriculture insecticides such as lead hydrogen arsenate and wood preservatives for instance chromated copper arsenate (CCA) increases arsenic levels in the environment (NZFSA 2005; Reilly 1980). Toxicity of arsenic to insects, bacteria and fungi led to its use as insecticides and wood preservatives.

Arsenic is readily soluble in water (solubility 20 g/L at 25°C) and its level is considerably high in aquatic region as compared with land (FSA 2005). Aquatic organism also has the ability to accumulate arsenic from the environment (WHO 1981) which makes the aquatic organism especially

fish as a common source of arsenic contamination for those higher in the food chains, such as humans.

Solid samples such as fish tissue must be transformed to liquid phase and undergo digestion whereby the organic components are destroyed, by acid releasing the metals that are dissolve into solution so that it can be analysed spectroscopically. Several conventional methods can be adapted to measure total arsenic in fish tissue. For example dry ashing where solid sample is placed in furnace at 500°C with $MgO/Mg(NO_3)_2$ (Vilano & Rubio 2001). This method provides total decomposition of organic matter, however, it is tedious, time consuming and causes loss of total arsenic. Another method that is usually adopted is acidic digestion such as the method by the Environment Protection Agency (EPA), method 3050B using HNO_3 or mixture of several concentrated acids of HNO_3/H_2O_2 are added and the mixture is heated up to 95°C**.** This open digestion system was reported as slow (Nam et al. 2010), requires more acid to affect full digestion and gives out dangerous nitrogen oxides (NOx) fumes, with poor recovery due to volatility arsenic at the required temperature. Therefore, the use of simple, fast and suitable extraction method which overcomes all the above setbacks in monitoring total arsenic in fish tissue is critical and has a great importance among the environmental studies.

Microwave assisted acid digestion procedures are gaining popularity recently due to the speed of the digestion process as well as less possibility of contamination during the process (Soylak et al. 2004). This is a closed system and the closed vessel can achieve higher temperature and pressure. The critical parameters in this procedure are the digestion temperature, time programme and the type of chemical used (Souza et al. 2007). Temperature is a critical element in acid digestion that will ensure complete decomposition of organic matter and provide clear solution. Microwave digestion system allows real time temperature specification (usually around 200°C) that is required to increase the boiling temperature of the acid, which will ensure the digestion of the sample. A variety of acid mixtures have been used for the extraction processes, (HNO_3-H_2O_2, HNO_3-HF, HNO_3-HCl) as chemicals, which depends largely on the nature of the matrix to be decomposed (Nam et al. 2010). Hydrochloric acid. (HCl), is useful for salts of carbonates, phosphates, some oxides and some sulfides. However, chloride in HCl will interfere with arsenic in ICP-MS; make HCl less favourable in arsenic analysis. Nitric acid, HNO_3 (boiling point 122°C) makes an oxidizing attack on many samples not dissolved by HCl. Hydrogen peroxide, H_2O_2 (boiling point 150°C) is a strong oxidizer which used together with HNO_3 to dissolve matters that is not fully decomposed by HNO_3.

Method validation is a vital component to allow it to produce reliable analytical data (ISO 17025 2005). It has become a basic pre-requisite for those laboratories that work in the official food control. By applying methods validated according to common procedures and performance criteria, the quality and comparability of the analytical results can be ensured (D'Ilio et al. 2008).

In this paper, four microwave assisted acid digestion methods were evaluated by allowing comparison among the digestion temperature, time programme, pre-treatment of sample and the chemical ($HNO_3/H2O_2$). Standards additions technique was employed for analysis of total arsenic extracted from fish, using ICP-MS. The methods were then validated using method performance criteria for chemical analysis which includes limit of detection (LOD), limit of quantification (LOQ), linearity and working range, precision and recovery.

Materials and Methods

SAMPLES

This method was developed and validated for fish tissue analysis, thus a blank sample should be chosen whereby it should match the actual sample matrix of this study. Catfish, *Clarias batrachus*, freshwater fish was chosen as the blank sample for the method validation from this point forward. Studies have shown that freshwater fish contribute to lower total arsenic contamination as compared with marine fish (Cheung et al. 2008; Ciardullo et al. 2010; Williams et al. 2009). The catfish was used in this study was obtained from a cultured and controlled environment and feeding to minimize the contamination. Preliminary study shows that the total arsenic level in catfish was lower than LOD.

The fish sample preparation for total arsenic analysis was according to The British Standard (2002). The fish was brought to the lab and the scales, fins, guts and roes were discarded and only the edible portion were utilized for the analysis. Fish fillet (tissue) was carefully removed from the fish bone without any contact with guts and roes. This is to prevent any contamination being introduced to fish tissue which may alter the existing level of total arsenic.

Fish muscle tissues samples were then freeze-dried in freeze-drying system (Lab-Conco, Model 79480, 77520, Kansas City, Missouri, USA) following the method of Abdul et al. (2010). The dried samples were ground using mortar and pestle and the powdered samples sieved through 0.8 μm mesh. The freeze-dried powder was then stored in amber glass bottle (as arsenic is light sensitive), prior to analyses at room temperature.

Certified reference materials, DORM-3 (dry fish tissue) were obtained from National Research Council (NRCC, Canada) used for development and validation of the method.

CHEMICALS

All chemicals used were of the highest purity and all solutions were prepared in distilled deionized water (DIW) obtained from Milli-Q Element system (Millipore, Bedford, MA, USA). An arsenic stock solution of 1,000 mg/L was obtained from Perkin Elmer (Connecticut, USA). The arsenic spike solution of 50 μg/L was prepared by diluting stock solution in DIW. Rhodium standard solution (1,000 mg/L) (Perkin Elmer, Connecticut, USA), was used

as internal standard to determine any drifts in the analysis. Rhodium was selected as internal standard for arsenic because its ionization energy (7.45 eV) is comparable with arsenic (9.78 eV). Among the concentrated acids, 65% (w/v) HNO_3 and 40% (v/v) H_2O_2 were provided by Merck, Darmstadt, Germany.

INSTRUMENTS

Ten vessels were mounted in closed-vessel microwave digestion system (Ethos 1-Milestone, Bergamo, Italy) and four were used for digestion. Total arsenic was determined by using ICP-MS (ELAN® DRC-e, Perkin Elmer SCIEX, Waltham, MA, USA), equipped with nickel cones and cyclonic spray chamber as a sample introduction system and using dynamic reaction cell mode with oxygen gas to remove polyatomic interference that may occur with the presence of argon and chlorine. The operating conditions of ICP-MS are given in Table 1.

METHOD PROCEDURES

The sample weight was 0.3 g with final volume of 25 mL and these parameters was fixed for all four extraction methods. The chemical and its consumption for each method can be referred to Table 2. One blank fish sample, 1 reagent blank and 3 replicates of standard addition of total arsenic in fish samples were analyzed for each method. The reagent blank concentration was then subtracted from the measured concentration in each digestion to give the final reported concentration of arsenic.

Four microwave assisted acid digestion procedures were compared namely method A, B, C and D. Extraction method A and B were referred with slight modification to Hirata et al. (2006) and Ashoka et al. (2009) study, respectively. The extraction methods C and D were modified from Milestone (2006) cookbook which specifies for fish sample. Each method offers different extraction

TABLE 1. Instrumental operating conditions of ICP-MS system

ICP-MS parameter	Operation conditions
Spray chamber	Cyclonic spray chamber
Sample introduction	Meinhard nebulizer
RF power	1400 w
Plasma ar flow	18 L/min
Injector	Alumina
Monitored ion m/z	91 (75As16O) and 103(Rh) for DRC
Dwell time	500 ms
Total acquisition time	600 sec
Oxygen flow for DRC	0.25 mL/min

TABLE 2. Steps of microwave heating programmes and acid digestion procedure for method A to D

Method	Variables					
	Pre-treatment	Chemical reactives and consumptions	Programme step	Time (min)	Power (W)	Temp (°C)
A	Kept at room temperature	65% HNO_3 (5 mL)	1	2	250	-
(Hirata et al. 2006)	for 15 min after addition	40% H_2O_2 (2 mL)	2	0.5	0	-
(Total run time: 28.5 min)	of chemical reactive before	(Total reactive volume: 7 mL)	3	5	300	-
	digestion		4	0.5	0	-
			5	5	450	-
			6	0.5	0	-
			7	5	600	-
			8	10	0	-
B	Kept at room temperature	65% HNO_3 (3 mL)	1	5	580	180
(Ashoka et al. 2009)	overnight after addition of	40% H_2O_2 (2 mL)	2	10	470	180
(Total run time: 45 min)	chemical reactive before	DIW (3 mL)	3	30	0	
	digestion	(Total reactive volume: 8 mL)				
C	None	65% HNO_3 (7 mL)	1	10	1000	200
(Milestone 2006)		40% H_2O_2 (2 mL)	2	25	1000	200
(Total run time: 35 min)		(Total reactive volume: 9 mL)				
D	None	65% HNO_3 (7 mL)	1	10	1000	200
(Milestone 2006)		40% H_2O_2 (1 mL)	2	15	1000	200
(Total run time: 25 min)		(Total reactive volume: 8 mL)				

condition and microwave programme. The variables that were tested include pre-treatment of sample, digestion temperature, time programme, chemical and its consumptions (HNO_3 and H_2O_2). Method A and B requires pre-treatment of sample where the sample are kept for 15 min and overnight after addition of chemical, while pre-treatment step were not required in method C and D. In method A, the temperature was not monitored as the temperature probe was not used as indicate by Ashoka et al. (2009). The digestion temperature for method B, C and D was 180 and 200°C, respectively. Total run time and chemicals for all four methods were given in Table 2.

The method development for this digestion method was carried out by studying the trueness (recovery) and precision (CV %). This was carried by standards addition of total arsenic in fish sample in replicates and recovery of total arsenic was calculated.

STATISTICAL ANALYSIS

The recoveries of the four extraction methods were analyzed using one way ANOVA to determine any significance difference at 95% confidence limit. The statistical analysis were carried out using SPSS software 11.5 2002 version. The accepted recovery range for standard addition is 80-120% and coefficient variation (CV) is as accordance to Commission Decision (2002) recommendation to select the most suitable or accepted digestion method.

METHOD VALIDATION

Method validation was conducted by evaluating limit of detection (LOD), limit of quantification (LOQ), linearity and working range, precision and recovery.

LIMIT OF DETECTION (LOD)

The approach that was employed is based on linearity study. In this approach the LOD was calculated based on standard deviation of the blank response or standard deviation of residual. A six level of standard solutions with concentration values between 0.08 to 1.17 mg/kg were prepared. The concentrations of standard were regularly distributed over the lower range of values. The standard solutions were analyzed at 3 different batches. The parameter was calculated for calibration curve. The LOD and LOQ were calculated using the formula below:

$$\text{LOD} = \frac{3 \times s_a}{b} \qquad \text{LOQ} = \frac{10 \times s_a}{b},$$

where s_a is standard deviation of the blank or y-intercept and b is sensitivity or slope of regression line. The LOQ value obtained through this calculation is a pre-determined basis. It is confirmed later in the following procedure.

LIMIT OF QUANTIFICATION (LOQ)

The LOQ obtained from above are confirmed in this procedure to ensure that the LOQ value obtained is achievable practically. A set of ten independent samples were spiked at the predetermined LOQ value. The signals of the spiked samples were measured and calculated using the following formula:

if $\frac{\left|LOQ - \bar{x}_{QL}\right|}{\frac{S_{QL}}{\sqrt{n}}} < 10$ then LOQ is considered to be valid, where $\bar{x}_{QL}$ is mean and S_{QL} is standard deviation.

LINEARITY AND WORKING RANGE

ICP-MS is distinguished for its wide linear concentration range. This parameter was evaluated, by examining the linear regression coefficient (R^2) of a calibration curve constructed with 10 standard solutions with concentration of 0.1, 0.5,1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/kg. These standards are analyzed in three different batches with freshly prepared standard solution in each batch.

PRECISION

The two most common precision measures for in-house method validation are repeatability and within-lab reproducibility. Repeatability was obtained when the analysis was carried out in one laboratory by one operator, with the same test method and material and using one piece of equipments within short period of time (within or between days precision). Precision within-lab reproducibility conditions in which test results are obtained with the same method on identical test items, with the same equipment and executed by different operators on long time interval.

In this study, repeatability (r) was evaluated by analyzing blank fish samples that were spiked at a minimum of three different concentrations of 0.2, 0.8 and 2.5 mg/kg (dry weight basis) that comprises the working range. Each concentration consists of a set of duplicate samples. These samples are analyzed at least in ten different batches by one analyst in one week interval time. Calibration solutions were prepared based on established linear range.

For within-laboratory reproducibility (R), the spiking concentration level and the calibration range protocol is similar to repeatability procedure. However, it was conducted by two different analysts with 4 different batches each with duplicate samples on different days on three month interval.

Horwitz approach that has been described in EURACHEM (2000) and Thompson et al. (2002) was used to evaluate the acceptance repeatability and within-lab-reproducibility. This approach involves the Horwitz equation to calculate the relative standard deviation and the value was used in HORRAT ratio which will be compared with acceptance values to finally evaluate acceptance of repeatability and within-lab-reproducibility.

Horwitz equation for the predicted repeatability relative to standard deviation is,

$$PRSD_r = C^{-0.15},$$

where, C is the concentration expressed as a decimal fraction.

Mean concentration, standard deviation and relative standard deviation for repeatability (RSD_r) and within-lab reproducibility (RSD_R) were calculated from the results, respectively. HORRAT ratio was compute from the data of calculated RSD_r, RSD_R and $PRSD_r$, $PRSD_R$ as shown below:

$$HORRAT_r(\text{Repeatability}) = RSD_r(\%) / PRSD_r(\%).$$

Acceptable values for this ratio are typically 0.5 to 2.

TRUENESS BY CRM

Trueness is close agreement between a conventionally accepted value or a reference value. The trueness of this method was validated by a certified reference material, DORM-3 (Fish Protein); from the National Research Council of Canada (Ottawa, Ontario Canada). The level of arsenic in CRM was 6.88 mg/kg. A set of ten replicates CRM with duplicate of each replicate were digested and analysed. An acceptance limit between 90 and 110% was selected in compliance with the CD No. 657/2002 (Commision 2002).

TRUENESS BY RECOVERY STUDIES

Blank fish sample and standards addition samples for minimum three different levels with concentration of 0.2, 0.8 and 2.5 mg/kg with (within linearity range including LOQ) at least 10 samples replicates were prepared. The sample was analyzed in duplicates. The mean value x for the 2 measurement x_1 and x_2 were calculated. The percentages of recovery were calculated:

$$\%\ \text{Recovery} = \frac{\left(\begin{array}{l}\text{conc in standards addition samples}\\ -\ \text{conc in blank fish sample}\end{array}\right)}{(\text{conc of analyte added})} \times 100\%$$

Accepted the trueness value were in compliance with CD No. 657/2002 as shown in Table 3.

TABLE 3. Accepted value of trueness for quantitative methods

Concentration	Trueness
≤1 µg/kg	50% - 120%
> 1 µg/kg to 10 µg/kg	70% - 110%
≥10 µg/kg	80% - 110%

Source : Commision 2002

RESULTS AND DISCUSSION

METHOD PROCEDURES

The recoveries of the four extraction methods were exhibited in Table 4. It is observed that method A, B, C and D exhibit recovery of 115.5, 111.6, 101.8 and 100.7% of mean recoveries respectively and was found to be in the range of 80 to 120%. The coefficient variation (CV) (%) for all four digestion methods were well below 10%. In Commission Decision (2002) stated that for mass fraction below 1 mg/kg, CV (%) should be lower than 16%. The results obtained shows that all four digestion method fulfills the criteria for trueness (recovery) and precision (CV). However, extraction method D demonstrates the smallest CV (%) i.e. 0.6% and closest to true value of 100% as compared with other methods.

The recoveries of the four extraction methods were utilized for significance study using statistical analysis. It was found there is significant value of 0.005 ($p \leq 0.05$) estimated. This shows that all four extraction methods are significantly different between each other at 95% confidence limit.

A post-hoc multiple comparisons were conducted using new Duncan multi range test was carried out to select the most acceptable extraction method among the four extraction methods studied. The output of the statistical analysis is stated in Table 4 where there are no significant difference ($p \geq 0.05$) between method A and B. In addition, methods C and D exhibit no significant difference ($p \geq 0.05$) between each other. Nevertheless, methods A and B, are significantly different ($p \leq 0.05$) from method C and D. Since method D demonstrates the lowest CV (%) and closest recovery mean (%) value to 100%, methods A and B were rejected. Method D was finally chosen as the most suitable or acceptable method because of the shortest run time and utilizes smaller amount of acid. Thus, this extraction method is not only economical, it is also saves time.

Though, methods A and B had a pre-treatment step before the digestion, the methods did not assist in increasing the efficiency of the extraction method. In fact, with the addition of pre-treatment step, this digestion required longer time for the total analyses. In method B, 3 mL of DIW was added to reduce the vigorous oxidizing reaction between HNO_3 and H_2O_2. Although, this addition did not give any impact on the recovery of the total arsenic in this study, however, it was found successful by Ashoka et al. (2009). Methods C and D were of similar steps except for the total run time and consumption of reagents. The combination of HNO_3 and H_2O_2 provides excellent digestion aid for fish sample tissue without traces of foam that is usually found in HNO_3 and HCl combination (Soylak et al. 2004). In addition, the digestion temperature of 200°C was optimum for increasing the boiling temperature of the chemicals without experiencing analyte loss as compared to open digestion system (Vilano & Rubio 2001). For the acid digestion procedures, the extraction time and the total reagent consumption are of great importance.

TABLE 4. Recoveries and ANOVA analysis of four extraction methods

Method	A(n=3)	B(n=3)	C(n=3)	D(n=3)	Sig(n=3)
Mean recovery (%)	115.5[a]	111.6[a]	101.8[b]	100.7[b]	0.005 (p≤0.05)
SD	4.6	1.6	6.5	0.6	
CV(%)	4.0	1.5	6.4	0.6	

Different alphabet on each method's mean recovery indicates significant difference (p≤0.05)

METHOD VALIDATION

LOD shows the sensitivity of the instrument to detect total arsenic; however this level is not used as the decision limit. LOQ level was determined as a detection method limit and frequently used as a decision limit and it is usually 5 or 10 times higher than LOD. The LOD and LOQ for wet weight basis obtained from this study were 0.01 and 0.05 mg/kg, respectively, as displayed in Table 5. A study conducted by Vilano and Rubio (2001) utilized the hydride generation-atomic fluorescence detection was only able to achieve the detection of 0.4 mg/kg in seafood, which is about ten times higher than ICP-MS determination. This shows that other analytical techniques could hardly reach this very low quantification limit (D'Ilio et al. 2008). Souza et. al (2007) described a limit of detection of 0.05 ng/mL and estimated 10 µg/kg as a limit of quantification in samples based on 1 g aliquots of samples. These limits were smaller than those established in this paper. However, the authors determined the limits of detection and quantification as (xblank+3sblank) and (xblank+10sblank), respectively, where xblank was the blank aqueous solution mean and sblank as the blank standard deviation. The limits presented here were assessed experimentally by standard addition assessment with fish samples, providing more realistic limits for the method. When standard addition samples was used, the detection and quantification limits were assessed involving all the analytical steps and not only the detection step.

The linear range between 0.1 and 5 mg/kg is accepted as the working range. The linear regression, R^2 was evaluated as linearity indicator (Souza et al. 2007) is obtained as 0.998. The R^2 is the coefficient of determination which tells us how well the prediction equation fits the data. The value of R^2 suggests a good fit when it is closer to 1.0. In general it serves as a confidence limit. For confidence of 90%, then R^2 should be 0.90 or higher. It was also observed from this study that the calibration passes through zero as shown in Table 6.

For trueness study, CRM (DORM 3) were analysed using the extraction method D. The certified value for arsenic is 6.88 mg/kg. The CRM data obtained from the above-mentioned method is 6.99 mg/kg. The recovery of the CRM data to certified value was 101.4 ± 2.5%. This CRM recovery showed compliance to the CD No. 657/2002 (Commision 2002). Study carried by Ashoka et al. (2009) showed the trueness value of DORM 3 was between 96 and 99%, which is almost consistent with this study.

The predicted values of RSD_r lie between 6.9% and 10.1% for total arsenic as detailed in Table 7. The $HORRAT_r$ values calculated by equation using predicted $PRSD_r$ and calculated RSD_r estimates were between 0.6 and 1.2. $HORRAT_r$ value was between 0.5 and 2.0 of acceptance value which indicated that the method performance was satisfactory. Similar performance was observed for $HORRAT_R$ for within-lab reproducibility, where the value obtained was between 0.5 and 1.2 was judge satisfactory within limits of acceptability of 0.5 to 2.0 as stated in Table 8. These values indicate that the method is capable of producing results that are fit for purpose. It is also evident in this study that RSD_r is smaller than RSD_R, as proposed by Codex 2006 as one of precision criteria.

Horwitz equation and HORRAT values is now widely used as a benchmark for performance of analytical methods (González & Herrador 2007). It is now one of the

TABLE 5. LOD and LOQ of As in fish (in dry and weight) using selected extraction method

Sample	LOD (mg/kg)	LOQ (mg/kg)
Dry weight	0.07	0.22
Wet weight	0.01	0.05

TABLE 6. Linearity parameter of As in ICP MS

Parameter	Linear range(mg/kg)	Calibration linear equation	R^2	Pass through origin/not different from zero
Arsenic	0.1-5	y = 0.0074x + 0.002	0.998	YES

TABLE 7. Calculated RSD_r(%) and predicted $PRSD_r$(%) with HORRAT ratio for three level of concentration for repeatability

Average concentration, C, mg/kg	RSD_r(%)	$PRSD_r$(%)	$HORRAT_r$
0.2084	11.6	10.1	1.2
1.0308	4.4	7.9	0.6
2.6062	4.0	6.9	0.6

TABLE 8. Calculated RSD_R(%) and predicted $PRSD_R$(%) with HORRAT ratio for three levels of concentration for within-lab reproducibility

Average concentration, C, mg/kg	RSD_R(%)	$PRSD_R$(%)	$HORRAT_R$
0.2041	15.7	20.2	0.8
0.7970	8.2	16.4	0.5
2.2650	16.7	14.1	1.2

acceptability criteria for many of recent adopted chemical methods analysis of AOAC INTERNATIONAL, the European Union and other European organizations dealing with food analysis.

Although in this study, the HORRAT value was agreeable within acceptable limit for repeatability and within-lab reproducibility, it may not be always the case in method validation. Consistent deviations from the ratio on the low sides (value less than 0.5) may indicate unreported averaging or excellent training and experience; consistent deviations on the high sides (value more than 2.0) may indicat inhomogeneity of the test samples, the need for further optimization or training, operating below limit of determination or an unsatisfactory method (Poitevin et al. 2009).

CONCLUSION

Numerous studies have been developed to improve the most accurate methods for proper extraction of chosen elements. The advantages of short digestion time, less acid consumption and high extraction efficiencies in microwave digestion procedures make them preferable when compared with the conventional methods. The most suitable extraction method obtained in this study was method D. It utilizes ultrapure concentrated nitric acid (HNO_3) to concentrated hydrogen peroxide (H_2O_2) at a ratio of 7 mL: 1 mL with the lowest run time of 25 min and digestion temperature of 200°C with no pre-treatment. It demonstrates fast and economical analysis with acceptable accurate performance. This method successfully fulfils the performance criteria for chemical analysis and the requirements set in the regulations of the European Union and National Public Health Laboratory, SOP A03-005, method validation in chemical analysis (SOP 2008). Method D achieved low detection limit of 0.05 mg/kg, with the recovery of 101.4 ± 2.5% from the CRM (DORM 3) and excellent precision for total arsenic. This method can therefore be used for determinations of total arsenic in fish samples.

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