Sana'a University Journal of Applied Sciences and Technology https://journals.su.edu.ye/index.php/jast
Vol. 1 | No. 4 | Page 339 – 345 | 2023 | ISSN : 2958-9568
Doi: 10.59628/jast.v1i4.664

Validation and Determination of Mercury in Yemeni Common Canned Tuna Using Direct Mercury Analyzer

Mahfoudh M. AL-Hammadi[1 *], Anass A. Alnedhary[2], Abdualqawi A. Numan[3,4]
Fatima A. Murshed[1]. Ranya A.Alalie[1].
[1]Department of Chemistry, Faculty of Sciences, Sana'a University, Sana’a, Yemen.
[2]Department of Chemistry, Faculty of Education, Khawlan Branch, Sana’a University, Sana’a, Yemen.
[3]Department of Chemistry, Faculty of Applied Sciences, Taiz University, Taiz, Yemen.
[4]Department of Pharmacy, Faculty of Medical Sciences, Al Janad University for Science and Technology, Taiz, Yemen.
Corresponding author: *m.alhammadi@su.edu.ye

ARTICLE INFO
Article history:
Received: Oct 8, 2023
Accepted: Oct 17, 2023
Published: Nov, 2023

KEYWORDS
1. Mercury
2. Yemen
3. DMA
4. Canned tuna
5. Validation

ABSTRACT
The presence of Hg in canned tuna flesh is a major source of customer concern. Thus, the primary goal of this investigation was to evaluate total mercury levels in Yemeni canned tuna. Thirty-one samples of seven popular locally canned tuna brands were acquired from local stores in Sana'a. A Direct Mercury Analyzer was used to determine the mercury content. The linearity of the method was tested by assessing a series of mercury standards in triplicates at concentrations ranging from 20 to 500 ppb. The correlation coefficient for this method was 0.9993. The repeatability of the procedure was measured as intra-day variation and given as the relative standard deviation, which did not exceed 4.45%. This indicated the excellent precision of the method. Mercury recoveries from spiked actual canned tuna samples were between 96.61% and 100.49%, demonstrating excellent accuracy and an acceptable procedure for mercury measurement. The limits of detection and quantitation were 1.62 ppb and 5.4 ppb, respectively. The mercury content in 31 canned tuna samples was less than the 500 ppb standard imposed by the Yemeni Organization for Standards Metrology and Quality Control (YSMO).

CONTENTS

1. Introduction:

Canned tuna is a popular component of the human diet owing to its convenience, affordability, taste, and health benefits. Fish are low in cholesterol and a wonderful source of protein, omega-3 fatty acids, and vitamin D. Improved pregnancy outcomes and fetal growth have also been related to fish consumption. [1] Big-sized species of tinned tuna (e.g., albacore and yellowfin tuna) contain moderate levels of mercury (Hg), whereas small-sized species (e.g., skipjack) contain roughly one-third of the mercury levels of albacore and yellowfin tuna

[1,2]. It has been discovered that mercury levels almost always increase with tuna size and age [3].

In contrast, recent studies have established a link between heavy fish consumption and adverse health effects due to increased Hg concentrations and other contaminants in seafood. Approximately 80% of the consumption advisories presently issued for fish are due to mercury, which hinders nervous system development, particularly in infants and children [4,5]. The primary suspect is methylmercury, which constitutes approximately 90% of the mercury found in fish [6,7].

Several analytical methods have been adapted for the detection and quantitation of mercy in fish flesh, including liquid or gas chromatography coupled with highly sensitive and selective detectors, such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), atomic emission spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), and thermal decomposition and thermal amalgamation atomic absorption spectrometry (TDA AAS) [1].

However, the figures of merit of these methods are largely dependent on the level of mercury contamination and the sample matrix. Therefore, to achieve high-quality and dependable results, several sample preparation steps, such as digestion, liquid-liquid extraction, solid-phase extraction, cloud point extraction, and pre-enrichment, may be involved [8,9]. DMA is an excellent choice for directly assessing mercury in a variety of gas, liquid, solid, and environmental samples. Its favorable properties, such as sensitivity, speed, simplicity, low operational cost, high analytical throughput, and environmentally friendly and trustworthy findings, account for its popularity and widespread use in quality control laboratories [10].

To be effective, DMA-based chemical analysis must be carefully validated for each sample commodity. Typically, a certified standard material is used to generate a calibration curve. However, an aqueous standard solution and quality control process are recommended for use in an external calibration approach as part of method validation and verification [10,11]. A literature review revealed a dearth of information on the mercury content of locally manufactured canned tuna in Yemen. Another issue that both consumers and the government are concerned about is the absence of a database tracking mercury pollution in Yemeni food products in general, and canned tuna in particular. Therefore, this study aimed to ascertain the total mercury content of seven brands of locally manufactured canned tuna that people often buy and use.

2. Materials and methods

2.1 Reagents and Materials

A mercury standard solution 1000 mg/L in 10% HNO_3 was purchased from Perkin Elmer (USA). Hydrochloric acid 37% w/w extra pure reagent grade, ACS, Spain. Deionized water with a resistivity of 18.2 MΩ using Direct Q3-Millipore (USA).

2.2 Instrument

The total mercury concentration was determined in canned tuna samples using a direct mercury analyzer (DMA-80, Dual Cell, Milestone, Waltham, Sorisole, Italy). DMA-80 was equipped with a dual spectrophotometer cell and a silicon UV photodetector. A high-purity air compressor (Milestone, Italy) was used as the combustion and carrier gases. Nickel sample boats were pre-cleaned to avoid contamination using the following procedure: rinsed with deionized water (DIW), then dried and heated in the furnace up to 650 °C for 2 min. The DMA-80 analytical approach followed US-EPA method 7473 [12]. The samples were weighed into Nikel boats and loaded into an autosampler for analysis. The DMA-80 operating conditions for all analyses are shown in Table 1.

Table 1: DMA-80 Operation Parameters

Parameter	Setting
Dring	200°C for 90 Second
Decomposition	650°C for 120 Second
Decomposition	650°C for 90 Second
Catalyst Temp	600°C
Purge Time	60 Second
Amalgamation	900°C for 60 Second
Recording Time	60 Second
Air Flow	120 mL /min

2.3 Preparation of mercury standard solutions

Intermediate standard solutions of (10 and 1 ppm) were prepared from a stock solution of 1000 ppm mercury by suitable dilution and were used for the preparation of working standards.

Working standard solutions (20–500 ppb) were prepared by diluting the intermediate solutions appropriately. All mercury standard solutions were stabilized in 2% HCl.

2.4 Sampling Sites, Sample Collection and Sample Preparation

2.4.1 Canned Tuna Samples

Thirty-one samples of popular local canned tuna from seven different brands were acquired from neighborhood markets in Sana'a, Yemen. The samples were obtained on different manufacturing dates. The criteria provided by the Minamata Convention and other well-known organizations such as WHO and US-EPA were followed for sampling, sample preservation, and transportation [13]. The sampling date, location, and brands of canned tuna samples were recorded.

2.4.2 Canned Tuna Samples Preparation

The canned tuna samples were brought to the laboratory where they were opened, and the oil was squeezed out of the tuna samples. The bulk of the tuna sample was then fragmented and crushed into a fine homogeneous pasty condition.

2.5 Spiked Samples Preparation

Homogenized tuna paste (0.1 g) was placed on the sample boat and then spiked with various volumes (10–400 µL) of a 0.1 ppm standard solution to test the accuracy of the method used to determine the amount of mercury in spiked samples. The recovery was determined by comparing the differences between the spiked and unspiked samples.

2.6 Validation Study

The validation parameters of the method, including linearity, precision, accuracy, limits of detection, and quantification, were examined.

3. Results and Discussion

3.1 Method Validation

3.1.1. Method Linearity and Precision

By performing a triple analysis on a 0.1 g real sample that had been spiked with various concentrations of mercury standard (2 – 50 ng), the linearity of the method was assessed. The average of detector response was plotted against the mercury concentration in nanograms to create a calibration curve, as shown in Fig. 1. The detector response and mercury content were linearly correlated up to 50 ng, and high method linearity was shown by the R^2 value of 0.999. The accuracy of the procedure was also assessed and is presented as the %RSD, as shown in Table 2. The %RSD fell within the permissible range established by the AOAC for peer-verified techniques [14] and varied between 1.05 and 4.45%.

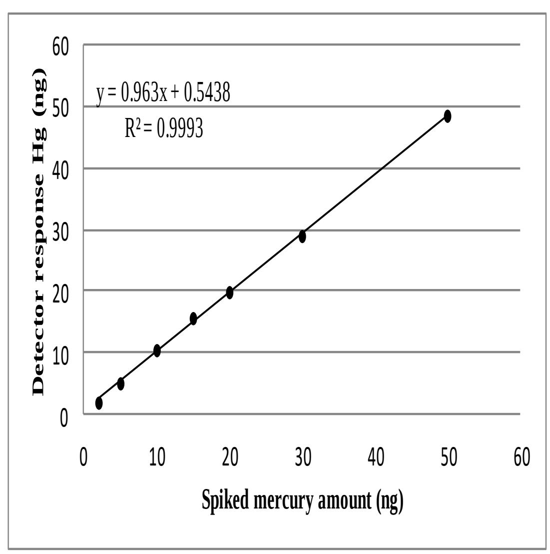

Figure. 1: Calibration Curve of Spiked Mercury.

Table 2: Method Validation Results of Mercury Analysis

Amount of Hg Spiked (ng)	Detector response (Measured Amount of Hg (ng))				SD	%RSD
	Replicate 1	Replicate 2	Replicate 3	Average (ng)		
2	1.9424	2.0665	1.8972	1.9687	0.0877	4.45
5	5.2398	4.9266	5.1662	5.1109	0.1638	3.20
10	10.5573	10.2927	10.6622	10.5041	0.1904	1.81
15	15.9747	15.8477	15.3156	15.7127	0.3497	2.23
20	20.2459	19.9859	19.5855	19.9391	0.3327	1.67
30	29.1259	29.3751	28.7080	29.0697	0.3371	1.16
50	49.1431	48.1184	48.6093	48.6236	0.5125	1.05

3.1.2. Accuracy (% Recovery)

Ten separate analyses of canned tuna samples injected with varying quantities of mercury standards (**1 - 40 ng**) were performed to determine the accuracy of the technique. Table 3 summarizes the recovery data, which varied from 96.61% to 100.49%. The obtained recovery values demonstrated the accuracy of the method. Furthermore, the estimated bias values varied from 0.29% to 3.39%, which is within the acceptable %RSD range for the peer-verified technique [14].

Table 3: Recoveries (% R) for Mercury in Canned Tuna Samples.

No.	Hg in unspiked sample, (ng)	Added volume in (µL) of 0.1 ppm Hg std.	Amount of added Hg, (ng)	Total amount of analyzed Hg (ng) (n=3)	Recovered amount of spiked Hg, (ng)	%R	%Bias
1	29.7156	10	1	30.6834	0.9678	96.78	3.22
2	29.7156	20	2	31.6953	1.9797	98.99	1.01
3	29.7156	40	4	33.7352	4.0196	100.49	0.49
4	29.7156	60	6	35.6478	5.9322	98.87	1.13
5	29.7156	80	8	37.5903	7.8747	98.43	1.57
6	29.7156	100	10	39.5218	9.8062	98.06	1.94
7	29.7156	150	15	44.7591	15.0435	100.29	0.29
8	29.7156	200	20	49.6203	19.9047	99.52	0.48
9	29.7156	300	30	59.2037	29.4881	98.29	1.71
10	29.7156	400	40	68.3584	38.6428	96.61	3.39

The limits of detection (LOD) and quantitation (LOQ) were calculated from the amounts of mercury in the spiked samples using the slope (S) and standard deviation (SD) of the calibration curve presented in Fig. 1.

$$LOD = 3 \times SD / S$$

$$LOQ = 10 \times SD / S$$

The calculated LOD and LOQ values were 0.324 ng and 1.08 ng, respectively, which are swell below the regulated amount of Hg in canned tuna (500 g/kg) as specified by various regulatory agencies, including the European Communities and the Joint FAO/WHO Expert Committee on Food Additives [15] and the Yemeni Organization for Standards Metrology and Quality Control YSMO [16]. This makes the Validated DMA-80 well suited for Hg detection in canned tuna meat.

3.2 Canned Tuna Samples Analysis

Thirty-one samples from seven different brands of local canned tuna obtained from Sana'a Yemeni markets were examined for mercury contamination. Table 4 summarizes the data for each brand (tuna type, number of samples, production site and date, size, and average Hg level in each individual brand). With an average concentration of 56.84 ± 1.64, the first brand manufactured in Almukulla city had the lowest content of Hg, while the highest concentration of Hg (387.85 ± 6.37) was identified in the yellow-canned tune of brand IV manufactured in Albidha Governorate.

The variance in Hg mean across the examined canned tuna brands might be impacted by the tuna's age, sex, fishing season, and location of the fish habitat [17].

Variation in Hg contamination also exists within the same type of meat, as shown in Table 4. The T-test calculated values at a confidence limit of 95% (P (0.05)) were highly significant between brands I and II (both were light tuna from the same manufacturer), and between brands III and IV (same type of white tuna, but different manufacturers); however, a T-test comparing the mean Hg content in brands VI and VII (both yellow tuna from the same facility) yielded insignificant results.

In general, the average Hg content in all examined brands (56.84 - 387.85 mg/kg) is lower than in canned tuna fish from Iran (0.79 mg/kg) [18], Morocco (0.5243 mg/kg) [19], Spain (0.5586 mg/kg) [20], Jordan (0.57 mg/kg) [21], and Saudi Arabia (0.86 mg/kg) [22]. Our findings, on the other hand, are consistent with those reported by Emami et al. for canned tuna samples from Iran [23].

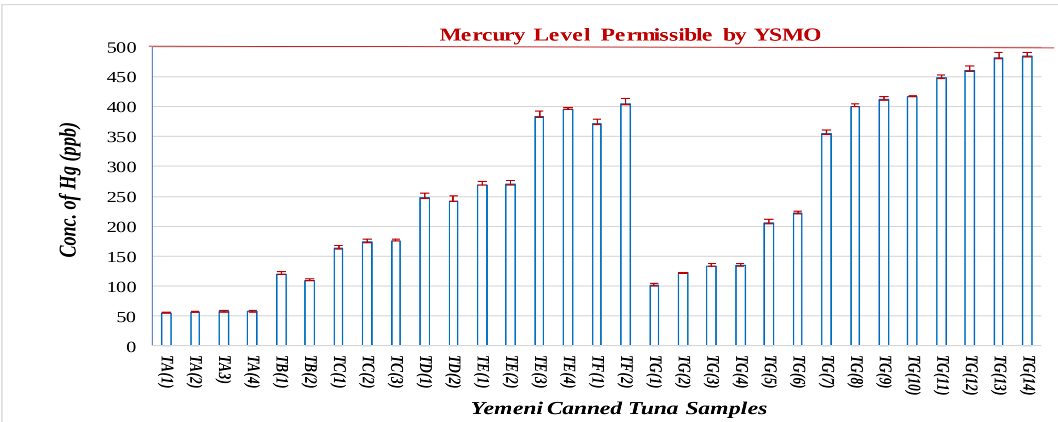

Figure. 2: Mercury Concentration in Yemeni Canned Tuna Samples

Table 4: Type of canned tuna samples

Brand	Type	n	location	Medium oil	meat shape in can	Product date	Average Hg conc.(ppb)	$T_{calc @95\%}$	T_{table}
I	light	4	Almukulla	sunflower	Small/soft	11.01.2020	56.84 ±1.46	0.00004	2.571
II	light	2	Almukulla	sunflower	Large/pieces	11.01.2020	114.88±3.14		
III	white	3	Almukulla	Soybean	Large/pieces	08.09.2020	171.16±4.01	0.000385	2.776
IV	white	2	Alshr	sunflower	Large/pieces	06.06.2020	244.7 ± 8.46		
VI	yellow	2	Albidha	soybean	Large/pieces	09.09.2020	387.8 ± 6.37	0.251931	2.131
VII	yellow	14	Almukulla	vegetable	Large/pieces	20.06.2020	312.7 ± 4.70		

Figure 2 shows the Hg concentration data for all 31 genuine canned tuna samples examined in this study. As can be observed, the greatest Hg concentrations were found in yellow tuna samples TG (13) and TG (14). Despite having a high concentration in comparison to the others, these two samples did not exceed the permitted limit of 500 ppb.

4. conclusion

A total of 31 common local canned tuna samples from seven brands were purchased from local markets in Sana'a City. The mercury concentration was determined using a Direct Mercury Analyzer (DMA-80). This method was fast, easy, simple, and rapid. This method has high linearity. The results were reported as %RSD which reflects the high precession of the method recoveries of mercury from spiked real samples were high accurate and appropriate method used. The mercury concentrations in the analyzed canned tuna samples were found to be below the legal limits specified by YSMO. This study improves baseline data and information on mercury concentrations in canned tuna in Yemen.

A total of 31 canned tuna samples from seven different brands were acquired from local stores in Sana'a, Yemen. A Direct Mercury Analyzer (DMA-80) was used to determine the mercury content. The procedure is quick, easy, sensitive, precise, and straightforward. Mercuric concentrations in canned tuna samples were determined to be within the permitted limits set by the YSMO. This study enhances Yemen's baseline data and information on mercury concentrations in canned tuna.

Acknowledgments

The authors would like to extend their gratitude to the Sana'a Water and Sanitation Local Corp (S.W.S.L.C) for allowing us to work in their laboratories and provide instruments and materials

5. References

[1] Esther .P; Marcelo .A; and Raquel. F. Cadmium, lead, tin, total mercury and methylmercury in canned tuna commercialized in São Paulo, Brazil. *Food Addit Contam* Part B **2017**; 1939-3229 http://dx.doi.org/10.1080/19393210.2017.1311379

[2] Cammilleri .G; Vazzana. M; Arizza .V; Giunta .F; Vella .A; and Dico .G . Mercury in fish products: what's the best for consumers between bluefin tuna and yellowfin tuna? *Nat Prod Res*,**2018**;1478-6427. http://dx.doi.org/10.1080/14786419.2017.1309538

[3] Kumar .G. Mercury Concentrations in Fresh and Canned Tuna: A Review. *Rev Fish Sci Aquac*,**2017**; 2330-8257 .https://doi.org/10.1080/23308249.2017.1362370

[4] Burger.J; and Gochfeld.M. Mercury in fish available in supermarkets in Illinois: Are there regional differences. M *Sci Total Environ*. **2006**; 367(2–3):1010–1016.

[5] Erstenberger .S; Artinson .A; and Ramer .J. An Evaluation of Mercury Concentrations In Three Brands of Canned Tuna. **2010**;29(2):237–42.

[6] Andrea R; Ornella .A ;Mery. M; Stefania .S; Maria. C; Silvia .B; Rosanna .T; Francesca. D; and Agnese .G, A Portable Setup for the Voltammetric Determination of Total Mercury in Fish with Solid and Nanostructured Gold Electrodes, *Molecules* **2019**, 24, 1910; doi:10.3390/molecules24101910

[7] Hajeb .P; Jinap .S; Ismail .A; Fatimah .A; Jamilah .B; and Rahim .M. Assessment of mercury level in commonly consumed marine fishes in Malaysia. 2009;20:79–84.

[8] Elodie .M; Nadia .O; Fabien .R; Robert .D; Catherine.B; André .M; Christophe. B; Joël. K; Damien .B;Jean .L; and Bruno. C, Modified 3D-printed device for mercury determination in waters, *Elsevier*,**2019**; https://www.elsevier.com/open access/userlicense/1.0/

[9] Suvi K, Elmeri L, Siiri P, Ari V, Determination of mercury at picogram level in natural waters with inductively coupled plasma mass spectrometry by using 3D printed metal scavengers, Analytica Chimica Acta,2019; https://doi.org/10.1016/j.aca.2019.09.075

[10] Fernández. R; Galán. P; Petit. M; Ordóñez. A; Loredo. J;and Rucandio. I.

[11] A rapid and robust method for the organic mercury determination in Hg mine waters, 9th International Mine Water Association Congress: 5-7 september **2005** , pp. 655–660

[12] Pourreza N and Ghanemi. K.. Determination of mercury in water and fish samples by cold vapor atomic absorption spectrometry after solidphase extraction on agar modified with 2-mercaptobenzimidazole. *J Hazard Mater*. **2009** ; 161 (2–3):982–987

[13] U.S. EPA. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis. Third Edition. Office of Science and Technology. Office of Water. U.S. Environmental Protection Agency. Washington, D.C. **2000.**

[14] U.S. EPA. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis. Third Edition. Office of Science and Technology. Office of Water. U.S. Environmental Protection Agency. Washington, D.C. **2000.**

[15] AOAC Peer-Verified Methods Program, Manual on policies and procedures, Arlington, Va., USA 1998. http://www.aoac.org/vmeth/PVM.pdf)

[16] Ebrahim, R; Mazyar, H; Hamid, R; Ali ,C; Amin, K; Mohammad, D; Mahdi, M; Abdol, E; Seyed, R; and Maryam, K, Analysis and determination of mercury, cadmium and lead in canned tuna fish marketed in Iran, *African Journal of Biotechnology* **2010**, Vol. 9(31), pp. 4938-4941

[17] YSMO Standardization for Canned Tuna (Arabic version), **2006**

[18] Hamba, S; Al – Haj, The artisanal tuna fishery in Yemen, *7th Expert Consultation on Indian Ocean Tunas, Victoria, Seychelles, 9-14 November*, **1998.**

[19] Sadighara, P; Mofid, V; and Mahmudiono, T, Concentration of heavy metals in canned tuna fish and probabilistic health risk assessment in Iran, *Int J Environ Anal Chem* **2022**, 1–11.

[20] Chahid, A; Mustaphab, H; Abdeljalil, B; Taoufiq, B, Heavy metals content of canned tuna fish:

estimated weekly intake, *Moroccan J Chem* **2015**, 3:1–3

[21] González, M; Martínez, J; and Fuentes, M, Mercury in canned tuna in Spain. Is light tuna really light?, *Food and Nutrition Sciences*, **2013,**Vol.4 No.7A, DOI:10.4236/fns.2013.47A007

[22] Ababneh, A; Al-Momani, I, Levels of mercury, cadmium, lead and other selected elements in canned tuna fish commercialized in Jordan, *Int J Environ Anal Chem*, **2013,** 93:755–766, http://dx.doi.org/10.1080/755766 03067319.2012.672981

[23] Ashraf, W, Levels of selected heavy metals in tuna fish, The Arabian Journal for Science and Engineering, 2006,31:8