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Determination of Total Mercury in Selected Seafood Samples Using Cold Vapor Atomic Absorption Spectrometry (CVAAS)

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Abstract

Mercury (Hg) is a persistent environmental contaminant that can accumulate in aquatic organisms and enter the human diet through seafood consumption. Monitoring total mercury concentrations in seafood is therefore important for food-safety assessment and environmental surveillance. This study aimed to determine and compare total mercury concentrations in selected seafood samples, including squid, prawn, fresh fish (mackerel), and canned fish, using cold vapor atomic absorption spectrometry (CVAAS) following microwave-assisted acid digestion. The analytical procedure was also assessed in terms of calibration linearity, recovery, and repeatability. Homogenized seafood samples (approximately 0.20 g) were digested with concentrated nitric acid using a closed-vessel microwave digestion system. Mercury was measured using a NIC RA-3000 Mercury Analyzer, with the standard addition method used to minimize matrix effects. The calibration curves demonstrated good linearity, with coefficients of determination (R^2) ranging from 0.9883 to 0.9956. The measured total mercury concentrations were 1.74 ppb in squid, 1.22 ppb in prawn, 5.69 ppb in fresh fish, and 6.92 ppb in canned fish. Recovery values ranged from 65% to 102%, while repeatability assessment for canned fish produced an RSD of 5.57%. Total mercury concentrations varied among the investigated seafood samples, with the highest measured value in canned fish and the lowest in prawn. However, the limited sampling design prevents statistical generalization, and the higher value in canned fish cannot be attributed to canning. Further studies should include larger representative samples, independent biological replicates, comprehensive analytical validation, and mercury speciation to better evaluate contamination and potential dietary exposure.

Keywords: Total mercury, seafood, fish, squid, prawn, canned fish, microwave digestion, mercury analyzer, standard addition method, food safety.

1. Introduction

Seafood, including fish, mollusks, and crustaceans, is an important source of high-quality protein and essential nutrients. However, these organisms can accumulate environmental contaminants, particularly heavy metals, making the monitoring of seafood safety an important public health concern. Mercury (Hg) is of particular interest because of its toxicity, persistence in aquatic ecosystems, and potential for bioaccumulation and biomagnification. Human exposure to mercury occurs primarily through the consumption of contaminated fish and seafood, particularly species occupying higher trophic levels (Hight & Cheng, 2005).

In aquatic environments, mercury occurs in several chemical forms and can be transformed into methylmercury, an organic form that readily accumulates in biological tissues and is transferred

through aquatic food webs. Consequently, mercury concentrations in seafood may vary according to species, habitat, feeding behavior, trophic level, age, size, and the anatomical tissue analyzed. Ritonga et al. (2022), for example, reported differences in total mercury concentrations among fish, cephalopods, and crustaceans, demonstrating the influence of biological and ecological characteristics on mercury accumulation.

Dietary exposure to mercury is an important pathway of human exposure. Recent large-scale monitoring has demonstrated the continued occurrence of elevated mercury concentrations in some seafood products, particularly predatory species such as swordfish, sharks, and tuna (Garofalo et al., 2025). These findings emphasize the need for reliable analytical methods and continued monitoring of mercury levels in foods intended for human consumption.

Cold Vapor Atomic Absorption Spectrometry (CVAAS) is widely used for mercury determination because mercury can be converted to volatile elemental mercury (Hg^0), which is subsequently measured at its characteristic wavelength of 253.7 nm. The technique provides suitable sensitivity for the determination of low mercury concentrations in biological and food matrices. Hight and Cheng (2005) demonstrated the applicability of CVAAS following microwave-assisted digestion for mercury determination in seafood, while more recent studies have confirmed its analytical performance when appropriate calibration, recovery assessment, and quality-control procedures are applied (Carbajo-Otero et al., 2023; Garofalo et al., 2025).

Accurate mercury determination also depends on effective sample preparation and control of matrix effects. Microwave-assisted acid digestion facilitates the decomposition of complex seafood matrices and improves the release of mercury prior to instrumental measurement. In addition, the Standard Addition Method can be used to compensate for matrix-related effects and improve the reliability of quantitative measurements, particularly when seafood matrices differ substantially in composition.

Despite extensive international research on mercury contamination in seafood, locally generated data remain important because mercury concentrations may vary with species, geographical origin, environmental conditions, and processing practices. Therefore, direct analysis of seafood products available for local consumption can provide useful information for food-safety assessment and future exposure studies.

Accordingly, the present study aimed to determine and compare total mercury concentrations in selected seafood samples, namely squid, prawn, fresh fish, and canned fish, using Cold Vapor Atomic Absorption Spectrometry (CVAAS). The study also evaluated the analytical response through calibration, recovery, and repeatability assessments and examined differences in mercury concentrations among the investigated seafood types.

2. Materials and Methods

2.1 Study Design

The study was conducted as an Experimental Analytical Laboratory Study to determine the concentration of total mercury in four selected types of seafood. Given the nature of the available data, the present study is considered a preliminary study, and the available data do not permit generalized statistical inference to all seafood products or to all samples within the investigated types.

2.2 Instruments and Equipment

Sample preparation and digestion were carried out using a MARS X Microwave Digester, equipped with polytetrafluoroethylene (PTFE) digestion vessels designed for acid digestion under closed-vessel conditions. The microwave system was used to decompose the organic matrix of the samples and convert the mercury present in the samples into a form suitable for subsequent analysis. Total mercury concentrations were determined using a NIC RA-3000 Mercury Analyzer, which operates based on reduction and vaporization to generate mercury vapor and measure it spectroscopically at a wavelength of 253.7 nm. The instrument requires a relatively small volume of sample solution; 5 cm^3 of the digested solution was used for each measurement. The auxiliary equipment used for sample preparation and analysis included

volumetric flasks with capacities of 50, 100, 500, and 1000 cm^3; micropipettes with a range of 100–1000 µL; graduated cylinders; beakers; bottles for storing deionized water; and an analytical balance capable of measurements to four decimal places.

a b c

Figure 1. (a) RA-3000 Mercury Analyzer; (b) MARS X Microwave Digester used for microwave-assisted sample digestion; and (c) digestion vessels used with the microwave digestion system.

2.3 Chemicals and Reagents

Analytical Reagent Grade chemicals and reagents were used throughout all stages of the chemical analysis. All solutions were prepared using deionized water to minimize the possibility of contamination or interference that could affect the measurement results. The chemicals and reagents used included mercury chloride ($HgCl_2$) as the mercury source and stannous chloride ($SnCl_2$) as the reducing agent for mercury vapor generation. Sulfuric acid (H_2SO_4) at approximately 96% concentration and nitric acid (HNO_3) at approximately 70% concentration were used for chemical treatment and digestion of the samples. Sodium chloride

(NaCl) was also used during sample preparation and treatment procedures, together with deionized water for the preparation of solutions and various dilutions.

2.3.1 Preparation of Stannous Chloride Solution

A 10% $SnCl_2$ solution was prepared by adding 50 cm^3 of sulfuric acid to a 500 cm^3 volumetric flask containing approximately 300 cm^3 of deionized water, while maintaining the mixture at a low temperature. Subsequently, 30 g of NaCl and 50 g of $SnCl_2$ were added. The mixture was shaken until complete dissolution, and the volume was then made up to the mark with deionized water.

2.3.2 Preparation of Diluted Sulfuric Acid

A 50% H_2SO_4 solution was prepared by slowly adding 250 cm^3 of concentrated sulfuric acid to a 500 cm^3 volumetric flask containing approximately 200 cm^3 of deionized water. The volume was made up to the mark with deionized water, and the solution was then allowed to cool before use.

2.3.3 Preparation of Diluted Nitric Acid

A 2% HNO_3 solution was prepared by adding 20 cm^3 of concentrated nitric acid to a 1000 cm^3 volumetric flask containing approximately 900 cm^3 of deionized water. The volume was then made up to the mark with deionized water.

2.4 Preparation of Mercury Standard Solutions

A 1000 mg/L mercury stock standard solution was prepared from mercury chloride ($HgCl_2$) by dissolving 0.1354 g of $HgCl_2$ in 100 cm^3 of deionized water, followed by gentle shaking until complete dissolution. An intermediate mercury standard solution at a concentration of 100 mg/L was subsequently prepared by transferring 10 cm^3 of the 1000 mg/L stock solution into a 100 cm^3 volumetric flask and making up the volume with 2% nitric acid (HNO_3). Another intermediate solution at a concentration of 1 mg/L was prepared by transferring 1 cm^3 of the 100 mg/L mercury solution into a 100 cm^3 volumetric flask and making up the volume with 2% HNO_3 solution. The 100 mg/L and 1mg/L intermediate standard solutions were freshly prepared prior to use. The 1mg/L mercury standard solution was used to prepare the calibration solutions, standard-addition solutions, and quality-control samples.

2.5 Cleaning of Glassware

The glassware used for sample and solution preparation was cleaned prior to analysis according to the procedure described in the report. The glassware was soaked in 10% nitric acid for 24 hours to remove any residues or contaminants that might accumulate on the glass surfaces. Following the soaking process, the glassware was rinsed with water before being used for the preparation of solutions and samples.

2.6 Sample Collection and Preparation

The study included four types of seafood: squid (A), prawn (B), fresh fish (Mackerel, C), and canned fish (D) from the Ayam Brand. The fresh samples were collected from a local market, while the canned fish samples were purchased from a local store. Approximately 500 g of each sample type was collected and transported to the laboratory for the necessary preparation and treatment procedures prior to analysis for the determination of total mercury content using Cold Vapor Atomic Absorption Spectrometry (CVAAS).

Figure 2: Homogenized samples for Mercury Analysis

2.7.Sample Preparation

Following collection, each sample underwent preliminary preparation to remove unwanted parts and obtain homogeneous tissue prior to digestion and analysis.

For the squid (A) sample, the head and other unwanted parts were removed. The edible portion intended for analysis was then placed in a blender and thoroughly homogenized to obtain a homogeneous sample representative of the original material.

For the prawn (B) sample, the edible muscular portion was separated and used for analysis after thorough homogenization using a blender.

For the fresh fish (Mackerel, C) sample, the head, gills, and bones were removed. The muscle tissue, particularly from the abdominal region, was collected as the portion used for analysis. The muscle tissue was thoroughly homogenized using a blender before the required amount was taken for the digestion procedure.

For the canned fish (D) sample, the liquid medium or sauce present in the can was removed, and the solid fish portion was used after thorough homogenization using a blender.

After completion of the homogenization process for all samples, the required amounts were taken for subsequent digestion and analysis to determine the total mercury content.

2.8 Sample Digestion Using Microwave Digestion

Acid digestion of the samples was performed using a MARS X Microwave Digester. Approximately 0.20 ± 0.01 g of each homogenized sample was accurately weighed directly into a PTFE digestion vessel.

Then, 4 cm^3 of concentrated nitric acid (HNO_3) was added to each sample. The acid was also used to rinse the inner walls of the digestion vessel to ensure that any sample residues adhering to the vessel walls were transferred into the digestion medium.

The digestion vessel was tightly sealed and placed in the rotor, after which the complete set of vessels was inserted into the microwave digestion system.

The digestion process was carried out at full instrument power for 5 min and 20 s, according to the operating conditions specified in the report.

After completion of the digestion cycle, the vessels were allowed to cool to room temperature. They were then transferred to a fume hood, where the vessels were carefully opened to release the pressure and remove the vapors generated during the digestion process.

The digested solutions were carefully transferred into 50 cm^3 volumetric flasks. The digestion vessels and their lids were rinsed with distilled water, and the rinsing solutions were added to the corresponding volumetric flasks to ensure maximum quantitative transfer of the digested material.

2.9 Calibration and Standard Addition

The Standard Addition Method was used to prepare the calibration curves in order to minimize matrix effects arising from the seafood samples during the determination of mercury concentration. A series of standard addition solutions was prepared using the 1 mg/L intermediate mercury standard solution. The final standard addition levels were 0, 5, 10, 15, and 20 ppb. Appropriate volumes of the mercury standard solution were added to the samples, and the volume was then adjusted with distilled water according to the analytical procedure. The volumes of the added mercury standard solution were as follows:

Table 1. Volumes of the added mercury standard solution used for the standard addition procedure.

Sample	Volume of 1 mg/L Hg Standard Solution (µL)	Final Added Concentration (ppb)
A1/B1/C1/D1	0	0
A2/B2/C2/D2	500	5
A3/B3/C3/D3	1000	10
A4/B4/C4/D4	1500	15
A5/B5/C5/D5	2000	20

After obtaining the measurement signals corresponding to each standard addition level, the relationship between the measurement signal and the added mercury concentration was plotted. Linear regression was then applied to estimate the mercury concentration in the sample from the intercept of the regression line with the concentration axis.

2.10 Quality Assurance and Analytical Quality Control

Quality Assurance/Quality Control (QA/QC) procedures were applied throughout the analytical process to assess the efficiency of the digestion procedure and the accuracy of the analytical measurements. For quality control purposes, a spiked sample was prepared for each analytical

batch using 0.20 ± 0.01 g of sample tissue. The sample was spiked with 0.5 cm³ of the 1 mg/L mercury standard solution prior to microwave digestion. The standard addition was performed before digestion to evaluate the efficiency of the sample preparation and digestion procedures and to verify the ability of the analytical method to recover a known amount of mercury added to the sample matrix. The recovery percentage (Recovery, %) was calculated by comparing the measured mercury concentration in the spiked sample with the mercury concentration in the unspiked sample and the expected concentration resulting from the added mercury standard, according to the following equation:

$$\text{Recovery}(\%) = \frac{C_{\text{measured}} - C_{\text{sample}}}{C_{\text{expected}}} \times 100$$

where $\mathbf{C_{measured}}$ represents the measured mercury concentration in the spiked sample, $\mathbf{C_{sample}}$ represents the mercury concentration in the unspiked sample, and $\mathbf{C_{expected}}$ represents the expected mercury concentration corresponding to the amount of mercury standard added. The recovery results were used as an indicator of the efficiency of the sample preparation and digestion procedures and the analytical performance of the method.

2.11 Determination of Total Mercury Using the Mercury Analyzer

A NIC RA-3000 Mercury Analyzer was used to determine the total mercury concentration in the digested samples. Before starting the measurements, the instrument was switched on and allowed to operate for 20 min to reach stable operating conditions. To minimize the possibility of interference or cross-contamination between measurements, the system was rinsed with deionized water before the analysis. Subsequently, 5 cm³ of either the standard solution or sample solution was transferred into the measurement tube. Then, 250 µL of 50% sulfuric acid (H_2SO_4) was added, followed by 250 µL of 10% stannous chloride ($SnCl_2$) solution. The tube was immediately sealed using the Impinger unit equipped with a glass bubbling tube. The assembly was then placed on the instrument holder, and the measurement was initiated. The measurement was based on the reduction of mercury-containing species in the acidic medium by $SnCl_2$, resulting in the formation of elemental mercury vapor. The mercury vapor was transported to the measurement cell, where the analytical signal was measured at a wavelength of 253.7 nm. After completion of each measurement, the Impinger unit was removed and rinsed with deionized water before the subsequent measurement to minimize the carryover of sample residues and reduce the possibility of cross-contamination.

2.12 Determination of Total Mercury Concentration

The total mercury concentration in each sample was determined using the standard addition method. A calibration curve was constructed for each sample type by plotting the instrument response against the concentration of added mercury. Linear regression was applied according to the following equation: ($y = ax + b$) . where y represents the measured analytical signal, x represents the added mercury concentration, a represents the slope of the calibration curve, and b represents the y-intercept. The mercury concentration in the sample was determined from the value of x corresponding to $y = 0$, which represents the x-intercept of the regression line. The absolute value of the x-intercept was then taken as the mercury concentration in the analyzed sample.

2.13 Assessment of Repeatability

Repeatability was assessed using the canned fish sample. Eight replicate measurements were performed under the same analytical conditions. The samples were weighed separately, and each sample was spiked with 500 µL of the 1 mg/L mercury standard solution prior to analysis. The arithmetic mean, standard deviation (SD), and relative standard deviation (RSD%) were calculated for the eight replicate measurements using the following equation:

$$\mathrm{RSD}(\%) == \frac{\mathrm{SD}}{\mathrm{Mean}} \times 100$$

According to the data reported, the mean measurement value was 10,387.38, with a standard deviation of 579.0406, resulting in an RSD of 5.57%. The RSD is used to assess the degree of agreement among replicate measurements performed under the same analytical conditions. A lower RSD value indicates lower dispersion of the measurements around the mean and, consequently, better repeatability of the analytical procedure.

2.14 Data Processing

The total mercury concentrations in the samples were calculated based on the standard addition curves and linear regression equations. The coefficient of determination (R^2) was calculated to evaluate the linearity of the calibration curves. Recovery percentages were calculated to assess the performance of the analytical procedure, while the mean, standard deviation, and relative standard deviation were calculated to evaluate measurement repeatability. According to the data reported, the R^2 values of the calibration curves were 0.9932 for squid, 0.9883 for prawn, 0.9925 for fresh fish, and 0.9956 for canned fish.

3. Results

Four categories of seafood and food samples were analyzed, including squid, prawn, fresh fish, and canned fish, to determine their total mercury content. To minimize the effects of the sample matrix on the analytical response, the Standard Addition Method was used to construct the calibration curves. The standard addition method involves adding known amounts of the analyte standard to equal volumes of the sample and measuring the resulting analytical response. The relationship between the concentration of added mercury and the measured absorbance (Abs) was represented using linear regression. The intercept of the regression line with the concentration axis was then used to estimate the amount of mercury originally present in the sample matrix.

3.1 Total Mercury Concentration in Squid Samples

The analysis of the squid samples showed a clear linear relationship between the added mercury concentration and absorbance over the concentration range investigated. As shown in Table 2 and Figure 6, the standard addition concentrations ranged from 0 to 20 ppb, while the absorbance values increased progressively from 0.054145 at zero added concentration to 0.562133 at an added concentration of 20 ppb. Linear regression analysis yielded the following calibration equation: $Y = 0.0253X + 0.044$ with a coefficient of determination of: $R^2 = 0.9932$. The high coefficient of determination indicates a strong linear relationship between the added mercury concentration and the analytical response, supporting the suitability of the linear regression model within the calibration range investigated.

Table 2. Added mercury concentrations, absorbance values, and sample weights used for the analysis of squid samples

Sample	Added Concentration (ppb)	Absorbance (Abs)	Sample Weight (g)
Std 1	0	0.054145	0.2014
Std 2	5	0.155151	0.2026
Std 3	10	0.309516	0.2051
Std 4	15	0.402276	0.2011
Std 5	20	0.562133	0.2005
QC (Spiked Sample)	5	0.126490	0.2034

As shown in ***Table2***, increasing the concentration of added mercury generally resulted in an increase in absorbance, which is consistent with the expected relationship between analyte concentration and spectroscopic response. ***Figure3*** also shows good agreement between the calibration points and the fitted linear regression line, as supported by the R^2 value of 0.9932. A high degree of linearity is important for demonstrating the applicability of the calibration curve to the estimation of mercury concentration in samples within the investigated concentration range. However, comprehensive evaluation of analytical performance should not rely on the coefficient of determination alone; it should also consider other validation parameters, including accuracy, recovery, limits of detection (LOD), and limits of quantification (LOQ).

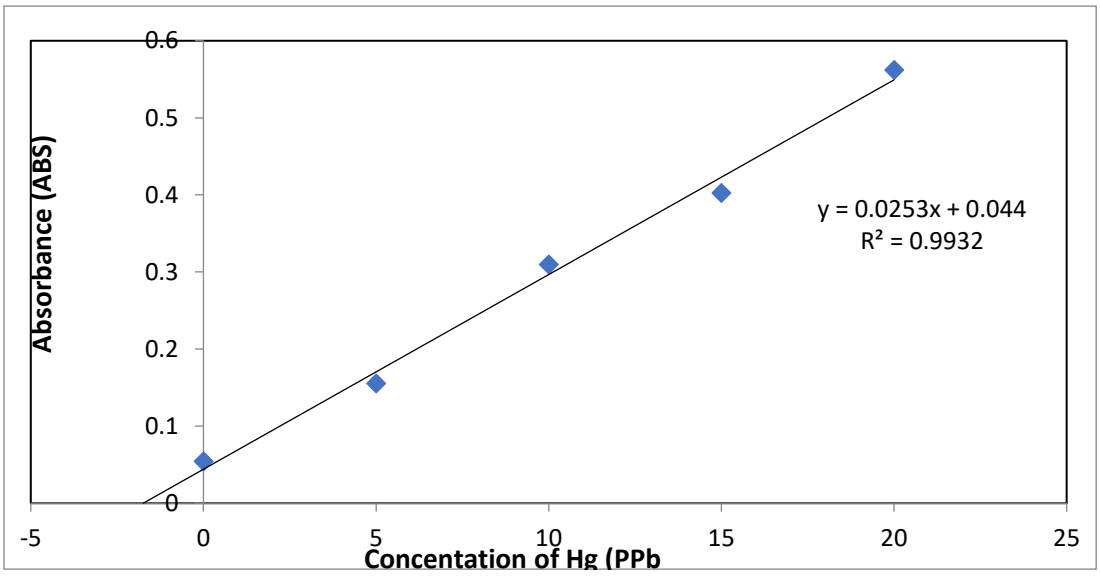

Figure 3. Calibration curve for the determination of mercury in the squid sample using the Standard Addition Method

The figure shows a strong positive linear relationship between the concentration of added mercury and the absorbance. As the mercury concentration gradually increased from 0 to 20 ppb, the absorbance increased clearly, indicating a good analytical response of the method.

The measurement points also show good agreement with the linear trend, with limited scatter around the regression line. This indicates good linearity and consistency of the analytical

response over the calibration range used. These findings support the applicability of the calibration curve for the analysis of squid samples and the estimation of their mercury content.

Overall, the results shown in the figure indicate that Cold Vapor Atomic Absorption Spectrometry (CVAAS) exhibited a good linear response for mercury determination. Therefore, the calibration curve can be used for quantitative estimation of mercury within the investigated concentration range.

3.2 Quality Control and Recovery Assessment

The performance of the analytical method was evaluated using a spiked sample to which a known concentration of mercury was added. The measured absorbance was 0.126490, while the added mercury concentration was 5 ppb.

The results showed that the calculated mercury concentration in the spiked sample was lower than the amount added, resulting in a recovery of approximately 65.2%. This finding indicates that a proportion of the added mercury was not recovered during the sample preparation or measurement procedures.

The relatively low recovery may be attributed to the loss of mercury during sample preparation or digestion, or to matrix effects associated with the squid sample, which may affect the efficiency of the analytical measurement. Therefore, the result suggests that matrix effects and/or sample preparation steps had a noticeable influence on the analytical performance.

Overall, the recovery result demonstrates that the method was capable of detecting and quantifying mercury; however, the recovery efficiency was moderate and may require further optimization, particularly with respect to sample preparation and minimization of matrix effects. The obtained recovery value should also be compared with the acceptable recovery range specified by the relevant analytical method or validation protocol before making a final assessment of the suitability of the method for quantitative analysis.

Based on the standard addition calibration curve, the x-intercept was 1.739 ppb, representing the equivalent mercury concentration in the sample solution used for calibration. The calibration curve also demonstrated good linearity, with a coefficient of determination of $R^2 = 0.9932$.

The quality control test yielded a calculated mercury concentration of 3.26 ppb, compared with an added concentration of 5 ppb, resulting in a recovery of 65.2%. This result indicates that the efficiency of the sample preparation and digestion procedures should be considered when interpreting the final mercury concentrations.

Accordingly, the value of 1602 ppb reported in the previous calculation was not included in the present results, because there is no valid mathematical basis for converting the value of 3.26 ppb to 1602 ppb using the sample weight alone.

3.3 Mercury in Prawn Samples

The total mercury content in the prawn samples was determined using the Standard Addition Method. The results showed a clear increase in absorbance with increasing concentrations of

added mercury. The absorbance increased progressively from 0.054971 in the absence of added mercury to 0.594316 at an added concentration of 20ppb, as shown in ***Table3*** and ***Figure7***. This consistent increase in absorbance indicates a clear analytical response to mercury over the concentration range investigated. The measured data points also showed good agreement with the fitted trend line, with relatively limited dispersion, indicating good linearity of the calibration curve. This was supported by the relatively high coefficient of determination, R^2 = 0.9883. Based on the standard addition calibration curve, the original mercury content in the prawn sample was estimated to be approximately 1.22ppb. This result indicates a relatively low mercury concentration in the analyzed sample.

Table 3. Added mercury concentrations, absorbance values, and sample weights for the prawn sample

Sample	Concentration (ppb)	Absorbance (Abs)	Sample Weight (g)
Std 1	0	0.054971	0.2051
Std 2	5	0.149948	0.2037
Std 3	10	0.292808	0.2041
Std 4	15	0.409627	0.2016
Std 5	20	0.594316	0.2076
QC (Spiked Sample)	5	0.141787	0.2055

As shown in ***Table 3***, the sample weights used to prepare the calibration points were relatively consistent, ranging approximately from 0.2016 to 0.2076 g, which minimizes the potential effect of differences in sample mass among the measurements.

The increase in added mercury concentration from 0 to 20ppb was accompanied by a clear increase in absorbance, which is consistent with the expected response of spectroscopic mercury measurements. The QC spiked sample also produced a measurable analytical response, indicating that the method was capable of detecting and recovering the mercury added to the prawn sample matrix.

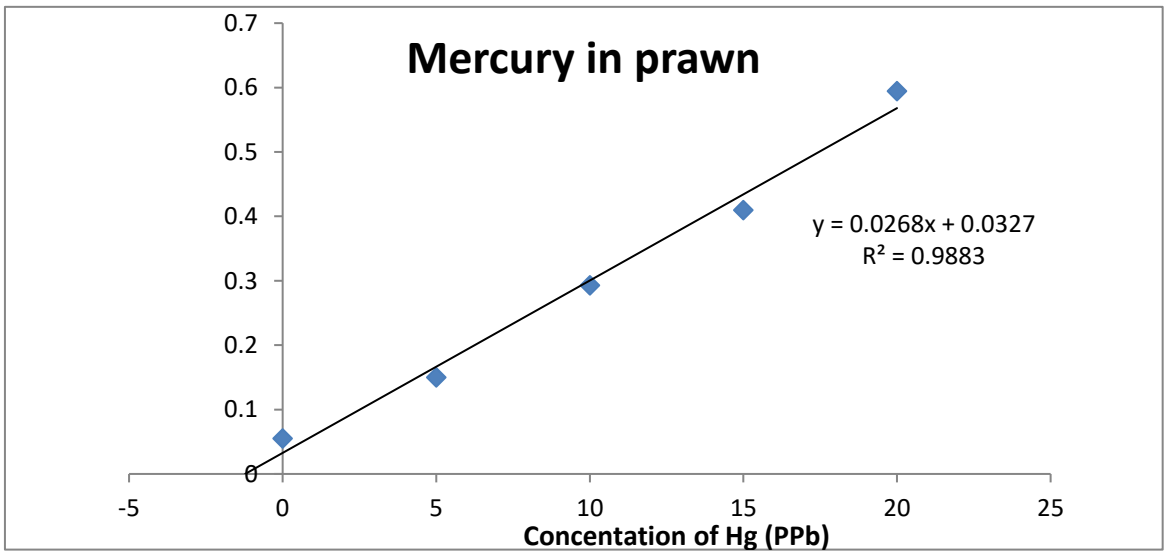

Figure 4. Calibration Curve for the Determination of Mercury in the Prawn Sample Using the Standard Addition Method

The calibration graph demonstrates a strong positive relationship between the added mercury concentration and absorbance; as the concentration of added mercury increased, the spectroscopic response increased clearly. The calibration curve also showed good consistency among the measured data points, supporting the suitability of the method for mercury determination in the prawn matrix. Regarding the quality control test, the recovery was 81.4%. This result indicates that a substantial proportion of the mercury added to the sample was recovered. At the same time, the recovery value suggests the possibility of partial mercury loss and/or matrix effects during sample preparation or measurement. Overall, the results obtained for the prawn sample demonstrated good linearity and an appropriate analytical response, together with a relatively low mercury concentration in the analyzed sample. The recovery of 81.4% provides an initial indication of analytical performance; however, it should be compared with the acceptable recovery limits specified in the analytical protocol or validation standard used in the study.

3.4 Mercury in Fresh Fish Samples

The total mercury content in the fresh fish samples was determined using the Standard Addition Method. The results showed a clear positive relationship between the added mercury concentration and absorbance. The absorbance increased progressively from 0.113436 at zero added concentration to 0.494152 following the addition of 20ppb mercury, as shown in Table 4 and ***Figure8***. This progressive increase in absorbance indicates a clear and consistent analytical response to mercury in the fresh fish matrix. The measured data points showed good agreement with the linear trend, with a coefficient of determination of $R^2 = 0.9925$, indicating a high degree of linearity of the calibration curve within the investigated concentration range. Based on the standard addition calibration curve, the total mercury concentration in the fresh fish sample was estimated to be approximately 5.688ppb. This value was higher than that obtained for the prawn sample, indicating variation in mercury concentrations among the seafood types investigated.

Table 4. Added mercury concentrations, absorbance values, and sample weights for the fresh fish sample

Sample	Concentration (ppb)	Absorbance (Abs)	Sample Weight (g)
Std 1	0	0.113436	0.2037
Std 2	5	0.208774	0.2091
Std 3	10	0.273796	0.2061
Std 4	15	0.390766	0.2019
Std 5	20	0.494152	0.2044
QC (Spiked Sample)	5	0.198687	0.2011

As shown in ***Table4***, the sample weights used for the calibration points were relatively consistent, ranging from 0.2019 to 0.2091 g, indicating that approximately similar amounts of sample were used for the analysis. A clear increase in absorbance was observed with increasing added mercury concentration. The absorbance increased from 0.113436 at 0 ppb to 0.208774, 0.273796, 0.390766, and 0.494152 at 5, 10, 15, and 20 ppb, respectively. This pattern confirms that the analytical response increased consistently with the amount of mercury added. The QC spiked sample produced an absorbance of 0.198687 following the addition of 5 ppb mercury, indicating that the added mercury could be detected in the fresh fish matrix.

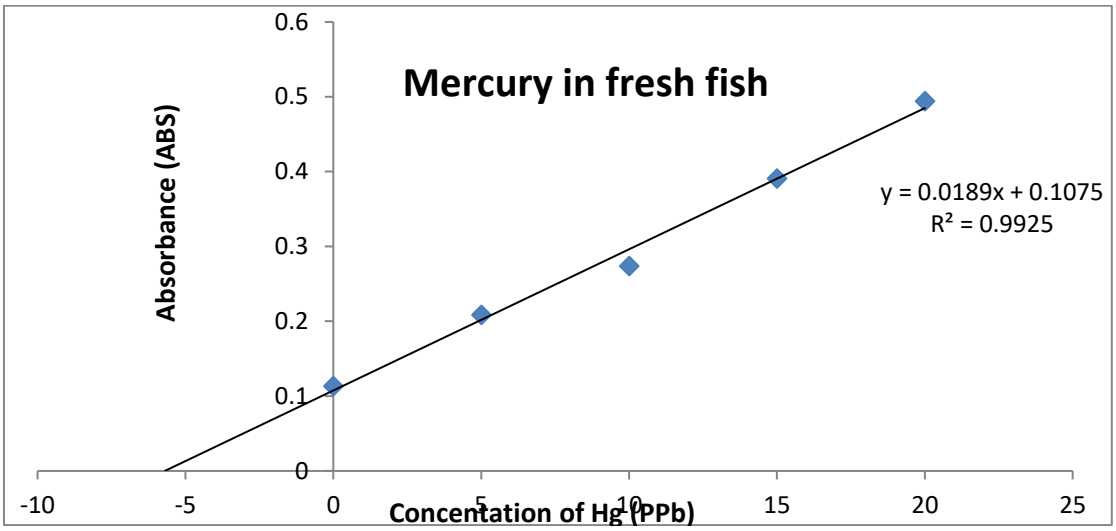

Figure 5. Calibration Curve for the Determination of Mercury in the Fresh Fish Sample Using the Standard Addition Method

The calibration curve showed a strong positive linear relationship between the added mercury concentration and the analytical response, with absorbance increasing clearly as the mercury concentration increased. The close agreement of the measurement points with the linear trend supports the quality of the calibration curve, consistent with the high coefficient of determination (R^2 = 0.9925). The recovery of the quality-control (QC) sample was approximately 96%, indicating a high recovery rate and good efficiency in recovering the mercury added to the fresh-fish matrix. This result also demonstrates good agreement between the added amount of mercury and the amount measured after sample preparation and analytical procedures. Overall, the fresh-fish samples showed very good linearity, a clear analytical response, and high recovery. The results indicate that the analytical method performed better in terms of recovery for this matrix than for the prawn samples, which may reflect differences in matrix effects or sample-preparation efficiency between the two seafood types. The fresh-fish sample showed a total mercury concentration of 5.688 ppb, with high calibration-curve linearity and a recovery of 96%. These findings support the suitability of the analytical method for the determination of mercury in this matrix under the conditions of the present study.

3.5 Mercury in Canned Fish Samples

The total mercury concentration in the canned-fish samples was determined using the Standard Addition Method. The results showed a clear increase in absorbance with increasing concentrations of added mercury. The absorbance increased from 0.133611 at 0 ppb to 0.519315 following the addition of 20 ppb mercury, as shown in ***Table5*** and ***Figure6***.

This consistent increase in absorbance indicates a clear analytical response to mercury in the canned-fish matrix. The measurement points also showed good agreement with the linear trend, with a coefficient of determination of R^2 = 0.9956. This high value indicates excellent linearity of the calibration curve over the investigated concentration range. Based on the standard-addition calibration curve, the total mercury concentration in the canned-fish sample was estimated to be approximately 6.915 ppb. This result indicates a measurable mercury content in the analyzed sample.

Table 5. Concentration, Absorbance, and Sample Weight of the Canned-Fish Samples

Sample	Added Concentration (ppb)	Absorbance (Abs)	Sample Weight (g)
Std 1	0	0.133611	0.2044
Std 2	5	0.223636	0.2051
Std 3	10	0.324959	0.2056
Std 4	15	0.398436	0.2086
Std 5	20	0.519315	0.2031
QC (Spiked Sample)	5	0.227270	0.2083

As shown in ***Table5***, the sample weights used for the calibration points were relatively consistent, ranging approximately from 0.2031 to 0.2086 g, indicating that comparable amounts of sample were used during the analytical procedure. The table also demonstrates a clear increase in absorbance with increasing concentrations of added mercury. The absorbance increased from 0.133611 at 0 ppb to 0.223636 at 5 ppb, followed by values of 0.324959, 0.398436, and 0.519315 at 10, 15, and 20 ppb, respectively. This trend confirms a consistent relationship between the amount of added mercury and the spectroscopic response. The QC spiked sample recorded an absorbance of 0.227270 following the addition of 5 ppb mercury, indicating a measurable response from the fortified sample and demonstrating the applicability of the analytical procedure for mercury determination in the canned-fish matrix.

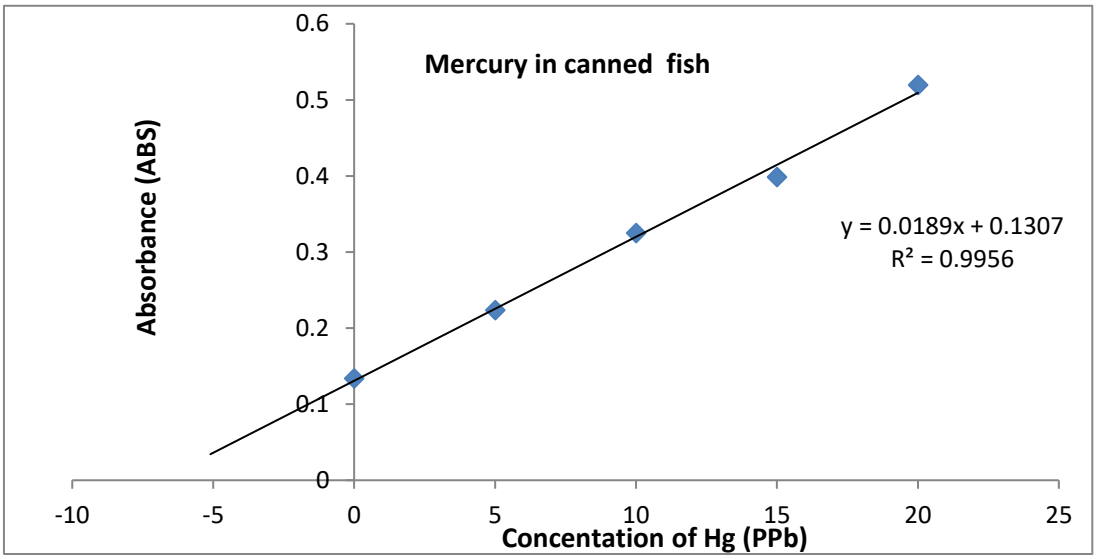

Figure 6. Calibration Curve for the Determination of Mercury in the Canned Fish Sample Using the Standard Addition Method

The canned-fish calibration curve showed a strong positive linear relationship between the added mercury concentration and the spectroscopic response, with good agreement between the measured points and the fitted line ($R^2 = 0.9956$). The estimated total mercury concentration was 6.915 ppb. The QC sample showed a recovery of 102%, indicating good recovery of the added mercury from the canned-fish matrix under the conditions of the present study. The slight deviation above 100% may reflect minor measurement variability or matrix effects.

3.6 Repeatability

The repeatability of the analytical method was evaluated using replicate canned-fish samples. Eight replicates were analyzed under identical operating conditions, with a known amount of mercury added to each sample.

Table 6. Total Mercury in Canned-Fish Replicate Samples

NO	Abs	Reading (ppb)	Weight (g)	Concentration (ppb)
1	0.543054	21.81767	0.206	10582
2	0.554098	22.40201	0.2075	10795
3	0.552272	22.3054	0.2089	10675
4	0.481446	18.55799	0.2048	9082
5	0.533021	21.28683	0.2066	10425
6	0.546634	22.00709	0.2043	10768
7	0.525421	20.88471	0.2077	10062
8	0.547163	22.03508	0.2054	10710
			mean	**10387.38**
			sd	**579.0406**
			rsd	**5.57%**

As shown in ***Table 6***, the eight replicate measurements showed relatively consistent analytical responses under identical experimental conditions. The mercury readings ranged from 18.558 to 22.402 ppb, while the absorbance values ranged from 0.481446 to 0.554098. The sample weights were also relatively consistent (0.2043–0.2089 g), indicating that variations in sample mass were unlikely to be a major source of the observed differences. The calculated standard deviation was 579.04, with an RSD of 5.57%. This relatively low RSD indicates limited dispersion among the replicate measurements and suggests acceptable repeatability of the analytical procedure under the conditions investigated. The general agreement between absorbance and mercury readings further supports the consistency of the analytical response. Overall, the repeatability assessment demonstrated that the method provided a relatively stable and consistent response for mercury determination in canned-fish samples, with an RSD of 5.57%.

4. Total Mercury Concentration in the Samples

Table7 summarizes the total mercury concentrations determined in the four investigated seafood categories. The mercury concentrations ranged from 1.22 to 6.92 ppb, with clear differences among the sample types. The lowest mercury concentration was recorded in prawn (1.22 ppb), followed by squid (1.74 ppb), whereas fresh fish and canned fish showed higher concentrations of 5.69 and 6.92 ppb, respectively.

Table 7. Summary of Total Mercury Concentrations and Recovery Percentages in the Investigated Samples

Sample Type	Mercury Concentration (ppb)	Recovery (%)
Squid	1.74	65
Prawn	1.22	81.4
Fresh Fish	5.69	96
Canned Fish	6.92	102

The results show that fresh and canned fish contained higher mercury concentrations than squid and prawn. This difference may be partly explained by variations in the biological and environmental characteristics of each species, including diet, age, body size, habitat, and trophic position within the food chain.

Methylmercury is one of the most important mercury species in relation to dietary exposure. It can accumulate in aquatic organisms through uptake from the environment and subsequent transfer through the food chain. As mercury moves between trophic levels, biomagnification may occur. Consequently, some predatory, larger, and longer-lived fish species tend to contain higher mercury concentrations than organisms occupying lower trophic levels.

Accordingly, the higher mercury concentrations observed in fish compared with prawn and squid may be associated with differences in trophic position and feeding behavior. However, fish species, body size, age, geographical origin, and other biological factors should also be considered before generalizing this interpretation.

With regard to canned fish, the highest mercury concentration observed in the present study was 6.92 ppb. This higher concentration cannot be attributed to the canning process alone based on the current data. It may primarily reflect the species of fish used for processing and its geographical origin, size, age, and mercury burden before processing. Although processing and canning may alter the composition of the sample and potentially affect contaminant concentrations, determining the specific effect of canning would require a direct comparison between the same fish species before and after canning.

Overall, the presence of mercury at different concentrations among the investigated samples highlights the importance of regular monitoring of mercury in seafood products, particularly in species that constitute an important component of the human diet.

4.1 Accuracy and Analytical Performance of the Method

The analytical performance of the method was evaluated using recovery and repeatability tests. Recovery percentages ranged from 65% to 102% in the spiked samples. Canned fish showed the highest recovery (102%), followed by fresh fish (96%) and prawn (81.4%), whereas squid showed the lowest recovery (65%).

These results indicate differences in mercury recovery among the investigated matrices. Fresh and canned fish showed relatively high recovery values, indicating good compatibility of the sample-preparation and measurement procedures with these matrices. In contrast, the relatively low recovery observed for squid may indicate matrix effects or partial analyte loss during sample preparation, digestion, or measurement.

The use of a freshly prepared standard solution for spiking and closed-vessel microwave digestion may have contributed to efficient sample preparation and reduced the potential for mercury loss during the digestion process.

Stannous chloride ($SnCl_2$) was used as a reducing agent to convert Hg^{2+} ions into elemental mercury (Hg^0), which is the form required for measurement by Cold Vapor Atomic Absorption Spectrometry (CVAAS). This reduction step facilitates the generation of mercury vapor and thereby provides an appropriate analytical response for mercury determination.

Regarding repeatability, the relative standard deviation (RSD) was approximately 5.57%, indicating relatively limited dispersion among the replicate measurements. The sample weights used in the repeatability assessment were also relatively consistent, supporting the consistency of the sample-preparation procedure among the replicates.

Overall, the recovery and repeatability results indicate that the analytical method demonstrated generally acceptable performance for most of the investigated matrices under the conditions of the present study. However, further optimization of the sample-preparation and digestion procedures may be necessary for matrices showing lower recovery, particularly squid.

5. Discussion

The present study demonstrated differences in total mercury concentrations among the four investigated seafood categories. Prawn had the lowest concentration (1.22 ppb), whereas canned fish showed the highest (6.92 ppb), followed by fresh fish (5.69 ppb) and squid (1.74 ppb). Such variation may be associated with differences in species, feeding habits, trophic position, age, body size, and environmental exposure. Mercury, particularly methylmercury, can bioaccumulate in aquatic organisms and biomagnify through the food chain, potentially resulting in higher concentrations in organisms at higher trophic levels.

From an analytical perspective, the calibration curves demonstrated good linearity over the investigated range, with R^2 values of 0.9883–0.9956. Recovery varied among the investigated matrices, ranging from 65% for squid to 102% for canned fish. This variation may reflect differences in matrix composition, digestion efficiency, or matrix-related effects. The relatively low recovery obtained for squid warrants further verification before the method can be considered adequately validated across all matrices. In contrast, the repeatability assessment for canned fish yielded an RSD of 5.57%, indicating relatively limited variation among replicate measurements under the conditions investigated; however, this result should not be generalized to the other seafood categories because repeatability was assessed using a single sample.

The results should also be interpreted in light of the fact that total mercury was determined rather than methylmercury separately. Therefore, the proportion of mercury present as methylmercury and its direct implications for dietary risk cannot be established from the present data alone. Although canned fish exhibited the highest measured concentration, this finding does not demonstrate an effect of the canning process itself, since differences in species, source, and other factors were not controlled experimentally.

Overall, the findings provide descriptive information on total mercury levels in the investigated seafood samples and demonstrate the applicability of the analytical approach under the conditions studied. However, the limited sample size, unclear number of independent biological replicates, and incomplete validation across matrices indicate that further studies with larger and well-defined sample sets and comprehensive method validation are required.

6. Conclusion

The present study demonstrated the applicability of Cold Vapor Atomic Absorption Spectrometry (CVAAS) combined with the Standard Addition Method for the determination of total mercury in different seafood products, with good linearity over the investigated concentration range. The total mercury concentrations in the investigated samples ranged from

1.22 to 6.92 ppb. The highest measured concentration was observed in canned fish, whereas prawn showed the lowest concentration. Recovery results varied among the investigated matrices, ranging from 65% to 102%, indicating that matrix characteristics and sample-preparation procedures may influence analytical recovery. The repeatability assessment produced an RSD of 5.57%, indicating relatively limited variability among replicate measurements under the analytical conditions used in the present study. Overall, the results indicate that the analytical procedure provides a potentially useful approach for the preliminary determination of total mercury in seafood samples, while emphasizing the importance of considering matrix effects and sample digestion and preparation procedures when interpreting the results. Future studies should include a larger number of samples from different geographical areas and sources, together with further optimization of sample-preparation procedures and more comprehensive analytical validation using Certified Reference Materials (CRMs) and additional quality-control parameters. It is also recommended that future investigations include mercury speciation analysis to determine the proportion of mercury present as methylmercury, in addition to comparing measured concentrations with applicable regulatory limits as part of a more comprehensive assessment of dietary exposure and potential health risks.

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