

# DEVELOPMENT OF A NEW TECHNIQUE FOR DETERMINATION OF MERCURY IN TAP WATER AND FISH MUSCLE TISSUE SAMPLES USING Mo-COATED TUNGSTEN-COIL ATOM TRAP COLD VAPOR ATOMIC ABSORPTION SPECTROMETRY

Muhammet ATASOY\*, Muğla Sıtkı Koçman University, Muğla Vocational School, Chemistry and Chemical Treatment Technologies Department, Chemistry Technology Program, Mentеше-48000 Muğla, Turkey, muhammetkarabas@yahoo.com

 <https://orcid.org/0000-0003-4312-1876>

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\*Corresponding author

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## Abstract

In this study, a new and low-cost technique for the determination of ultra-trace level of total mercury (T-Hg) was developed by combining a Mo-coated tungsten-coil (W-coil) placed in a quartz tube atomizer for in situ trapping of gaseous mercury with a conventional cold vapor atomic absorption spectrometry (CV-AAS). The optimum operating conditions of the parameters in the proposed technique were determined experimentally in order to achieve lower detection limits. The analytical results obtained from the developed approach were compared with those obtained from the traditional CV-AAS technique. The developed technique demonstrated a significant 39.7-fold improvement in sensitivity compared to the traditional technique, as expressed by the characteristic concentration ( $C_0$ ). The technique described yielded a limit of detection (LOD) of  $0.017 \mu\text{g L}^{-1}$  and a relative standard deviation (RSD%) of 3.6% for a capture time of 120 s. The proposed analytical procedure was utilized to determine the concentrations of Hg in certified reference materials, spiked tap water samples and fish muscle tissue samples. The recovery values for the water samples were satisfactory, ranging between 104.9% and 111.3%.

**Keywords:** Mercury determination, Tap water samples, Fish samples, Atom trap, Mo-coated W-coil

## Mo-KAPLI TUNGSTEN-SARMAL ATOM TUZAK SOĞUK BUHAR ATOMİK ABSORPSİYON SPEKTROMETRİSİ KULLANILARAK MUSLUK SUYU VE BALIK KAS DOKUSU ÖRNEKLERİNDEKİ CIVA TAYİNİ İÇİN YENİ BİR TEKNİĞİN GELİŞTİRİLMESİ

### Özet

Bu çalışmada, gaz halindeki cıvanın yerinde tuzaklanması için bir kuvars tüp atomlaştırıcı içine yerleştirilen Mo-kaplı tungsten-sarmal (W-sarmal) ile geleneksel soğuk buhar atomik absorpsiyon spektrometresi (CV-AAS) birleştirilerek ultra eser düzeyde toplam cıva (T-Hg) tayini için yeni ve düşük maliyetli bir teknik geliştirildi. Önerilen teknikteki parametrelerin optimum çalışma koşulları, daha düşük analiz seviyelerine inebilmek için deneysel olarak belirlendi. Geliştirilen teknikten elde edilen analitik sonuçlar geleneksel CV-AAS tekniğinden elde edilenlerle karşılaştırıldı. Geliştirilen teknik, karakteristik konsantrasyon ( $C_0$ ) ile ifade edildiği üzere, geleneksel tekniğe kıyasla duyarlılıkta 39,7 katlık bir zenginleştirme elde edildi. Önerilen teknik ile 120 saniyelik bir tuzaklama süresi için tayin sınırı (LOD)  $0,017 \mu\text{g L}^{-1}$  ve bağıl standart sapma (RSD%) ise %3,6 olarak hesaplandı. Önerilen analitik prosedür, sertifikalı bir referans malzemeler, spike edilmiş musluk suyu örnekleri ve balık kas dokularındaki Hg konsantrasyonlarını belirlemek için kullanıldı. Su numuneleri için geri kazanım değerleri tatmin edici olup %104,9 ile %111,3 arasında değişmekteydi.

**Anahtar Kelimeler:** Cıva Tayini, Musluk suyu numuneleri, Balık numuneleri, Atom tuzak, Mo-kaplı W-sarmal

### Cite

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### 1. Introduction

Mercury (Hg) is widely recognized as one of the most hazardous heavy metals due to its extensive use in

industrial and agricultural sectors [1]. Even at very low levels, Hg is toxic to living cells and has no beneficial biological functions [2]. Hg can easily penetrate the living food chain and interact with the human central

nervous system, resulting in neurotoxic effects even at relatively low concentrations [3]. Hg can accumulate in the people bodies through consumed water. Therefore, the determination of Hg content in the drinkable water samples becomes very important [4]. The US EPA has presently established the maximum allowable level of Hg in environmental water at  $2.0 \text{ ng mL}^{-1}$  [5]. One of the main sources of mercury intake into the human body is the consumption of fish and fishery products [6]. Fish are a significant source of Hg for humans due to their ability to accumulate significant amounts of this element in their tissues [7]. The United States has established a maximum concentration of total mercury in fish at  $1.0 \text{ } \mu\text{g g}^{-1}$  (wet weight), while in Europe the maximum limit is  $0.5 \text{ } \mu\text{g g}^{-1}$  (wet weight) [8]. Sea bass (*Dicentrarchus Labrax*) and white seabream (*Diplodus sargus*) are among the most consumed fish species by the local people living in Muğla province. Therefore, it is imperative that the mercury concentrations in these fish species consumed in the Mugla province could be monitored on a regular basis. Nowadays, powerful techniques with high sensitivity are necessary today to detect ultra-trace levels of Hg in drinking water and fish samples. Various analytical methodologies have been developed to detect Hg at very low levels, but few of them have sufficient sensitivity [5]. The most common methods are cold vapor atomic absorption spectrometry (CV-AAS) [9], CV-AFS [10], ICP-OES [11] and ICP-MS [12]. ICP-MS is one of the most widely used methods for the detection of Hg due to its high sensitivity and selectivity [4]. However, due to the high cost of both instrumentation and operation, it is inaccessible to many researchers [13]. The use of cold vapor (CV) in AAS has emerged as a favored approach for mercury analysis due to its remarkable sensitivity and utilization of straightforward and cost-effective equipment and chemicals [14]. Moreover, CV-AAS offers significant advantages, such as separating the analyte from the matrix, efficiently transporting analyte atoms to the atomizer, and utilizing easy and economical instrumentation [15]. ICP-OES and CV-AAS are commonly used procedures for the analysis of trace levels of Hg, but very low levels of Hg in water and food samples do not fall within the analytical limits of these methodologies [5]. Therefore, pre-enrichment techniques are needed to obtain accurate, reliable and sensitive results.

The sensitivity of the AAS method is enhanced by trapping volatile analyte species obtained through chemical vapor generation on a material surface just before analysis [16]. Various investigations have employed a graphite tube [17], a tungsten tube (W-tube) atomizer [18], and a tungsten coil (W-coil) surface [19] for in-situ capture of the volatile species produced. In certain studies, sensitivity was enhanced by coating the surface of the W-coil with elements such as Rh, Ir, Pt, Pd, or Au [8,18,20-21].

The objective of this research is to improve a low-cost and easy technique with high sensitivity for the analysis

of T-Hg concentrations in tap water and fish muscle tissue samples. As far as I know, this research is the first investigation to illustrate the capturing, enrichment and releasing of Hg using the Mo-coated W-coil CV-AAS methodology. The proposed technique consisted of first separating Hg as a volatile species from the sample matrix using cold vapor generation system. Subsequently, the volatile Hg atoms are pre-concentrated on a heated atom trap and then subjected to revolatilization before being transported to an unheated QTA. Optimization studies were conducted on the analytical parameters to obtain maximum trapping and releasing efficiency. The analytical performance of the described technique was compared with the traditional cold vapor methodology. Finally, the application of the technique was demonstrated by analyzing certified reference materials (CRMs) (NIST SRM 1641e mercury in water and dolt:5 dogfish liver), tap water samples and fish tissue samples.

## 2. Materials and Methods

### 2.1. Reagents

All reagents were supplied by Merck (Darmstadt, Germany) and were of at least analytically acceptable quality. Pure water obtained from the Milli-Q water purification system (Millipore, USA) was used to produce diluted solutions. A mercury(II) standard stock solution with a concentration of  $1000 \text{ mg L}^{-1}$  was used in experimental studies, and other mercury standard solutions were generated by diluting appropriate amounts from this stock solution.  $\text{NaBH}_4$  was chosen as the reductive agent and NaOH as the stabilizer. These two solutions were prepared inside each other and fresh daily. Suprapure purity HCl was used for the acid solution. Tap water samples were collected in the Muğla province of Turkey.

### 2.2. Instrument and Apparatus

The determinations were carried out using an Agilent 240 AAS (Agilent Technologies) equipped with a VGA 77 chemical vapor generation system. The rate at which the solutions were introduced into the chemical vapor generation system was modified by adjusting the tubing. To avoid interference from the ground, a deuterium lamp was used. For mercury analysis, the hollow cathode lamp, slit width and wavelength were set to 4.0 mA, 0.5 nm and 253.7 nm, respectively. QTA was affixed to the nebulizer using a standard cell holder without applying any flame. A small quartz tube measuring 50.0 mm in length was mounted at the tip of the QTA. The tube had a hole in the middle that was large enough for the W-coil to enter. The experiments were conducted using a W-coil extracted from a 15-volt, 150-watt floodlight bulb (HLX 64633, OSRAM, Germany). After treating the surface of the coil with Mo, it was mounted inside a small quartz tube. The temperature of the coil was adjusted using a manually adjustable power supply. The temperature values equivalent to the amperage values in the power supply

were determined by touching the tip of the coil with a thermocouple (Testo 925, Germany).

### 2.3. Coating Process

A 1000 mg L<sup>-1</sup> Mo solution was utilized in the coating process. A 20 µL droplet of the solution was applied to the coil surface. As per the study by Atasoy [22], the heating procedure of the coil included 3.8 A for 60 seconds, 4.2 A for 30 seconds, a brief interruption at 0 A for 5 seconds, and a surge to 7.0 A for 5 seconds, all repeated three times. During the deposition procedure, a stable flow rate was maintained for both H<sub>2</sub> and Ar, with levels set at 40.0 mL min<sup>-1</sup> and 300.0 mL min<sup>-1</sup>, respectively.

### 2.4. Sample Preparation Process

Sea bass (*Dicentrarchus Labrax*) and white sea bream (*Diplodus sargus*) were purchased from the fish market in Menteşe district of Muğla province. 0.5 g of muscle tissue from the each fish samples was taken. 0.5 g of Dolt:5 (dogfish liver) certified reference material was also weighed. Then, 10 mL of concentrated HNO<sub>3</sub> was added to the samples and digested using a microwave system (CEM, Mars 6).

### 2.5. Analytical Process

The performance of the developed technique was compared to the cold vapor method to better demonstrate the effectiveness of the developed technique. Peak height was measured throughout all studies. Optimal values of the parameters that can affect signal stability and height were determined for both methods. The initial step was to identify the optimal parameter values in the conventional approach. The proposed technique was based on these parameters, and the remaining variables of the proposed approach were optimized. The proposed method consists of two stages. At the capturing stage, firstly, the solutions were introduced to the system. The H<sub>2</sub> stream and the capturing temperature were adjusted to the optimal values. Volatile Hg species were captured on the trap surface for a period of time. In the stripping stage, primarily, the flow of solutions introduced to the system was interrupted. Subsequently, the H<sub>2</sub> stream and trap temperature were simultaneously increased for a few seconds to release the analyte atoms from the trap surface, resulting in a transient analyte signal. A schematic diagram of the developed technique is presented in Figure 1.

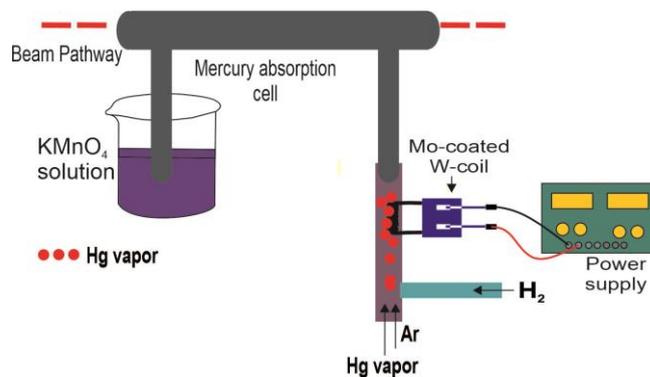


Figure 1. The schematic overview of the developed technique

## 3. Results and Discussion

### 3.1. Optimization of Parameters

The optimized parameters for the conventional cold vapor AAS method included concentrations of HCl, NaBH<sub>4</sub>, and NaOH also the Ar flow rate. During the optimization process, a univariate approach was employed, with the other parameters held at their optimal values. The stability and reproducibility of signals were taken into account when determining the optimum value of each parameter. The conventional method's parameters were optimized using a 10.0 µg L<sup>-1</sup> Hg solution. The optimal amounts of HCl, NaBH<sub>4</sub>, and NaOH were determined to be 5.0 mol L<sup>-1</sup>, 0.3% (w/v), and 0.5% (w/v), respectively, based on experimental studies. The best appropriate Ar flow rate was identified to be 100 mL min<sup>-1</sup>. Both the NaBH<sub>4</sub> and HCl solutions were injected into the system simultaneously, flowing at an identical rate of 4.40 mL per minute, whereas the analyte solutions were introduced at a rate of 5.35 mL per minute.

The proposed method's remaining parameters were optimized based on the optimum values of the variables in the conventional methodology. The optimized parameters in the developed technique include the collection and re-evaporation temperatures, the H<sub>2</sub> stream supplied to the system during the trapping and re-evaporation stages, and the trapping time. During the optimization of the parameters, a 2.0 µg L<sup>-1</sup> Hg solution was utilized.

The experiments showed that analyte atoms did not accumulate on the trap surface in the absence of external temperature in the trap system, resulting in no analyte signal. However, upon application of temperature to the trapping setup, the analyte signals began to increase. The study determined that the ideal collection temperature was 122 °C, while the ideal stripping temperature was 786 °C. During the stripping step, the analyte signal remained constant at temperatures above this value.

The amount of H<sub>2</sub> delivered to the system is a crucial parameter for the developed methodology. The optimum H<sub>2</sub> stream during the collection stage was determined to be 50.0 mL min<sup>-1</sup>.

If H<sub>2</sub> was not delivered to the system during this step, the analyte atoms could not be adequately deposited on the trap surface. It is important to maintain a small amount of H<sub>2</sub> during the collection step. However, excessive amounts of H<sub>2</sub> during collection caused a decrease in analyte signals. During the stripping step, H<sub>2</sub> stream was slightly increased. The optimal H<sub>2</sub> stream during this step was determined to be 110 mL min<sup>-1</sup>. Due to the cooling effect of H<sub>2</sub>, stream rates above this value were result in reduced re-evaporation efficiency.

Another crucial parameter is the collection time, as an increase in signals was observed with longer collection times. However, the observed increases were lower than anticipated for collection times exceeding 120 s. As a result, the optimal collection time was determined to be 120 s to minimize chemical usage. Table 1 summarizes the optimal parameter values.

Table 1. The most suitable parameter values for the proposed technique.

Parameter	Optimal values
Acidic solution	5.0 mol L <sup>-1</sup> HCl, 4.40 mL min <sup>-1</sup>
Reducing reagent	0.3% (w/v) NaBH <sub>4</sub> , stabilized in 0.5% (w/v) NaOH,
Analyte stream	5.35 mL min <sup>-1</sup>
Stream of gases in the capturing stage	100 mL min <sup>-1</sup> Ar; 50 mL min <sup>-1</sup> H <sub>2</sub>
Stream of gases in the re-evaporation stage	100 mL min <sup>-1</sup> Ar; 110 mL min <sup>-1</sup> H <sub>2</sub>
Capturing temperature	122 °C
Re-evaporation temperature	786 °C
Re-evaporation time	120 s

### 3.2. SEM-EDS Analysis

The surface structure analysis of both the uncoated and coated coil was performed by scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) at the Central Research Laboratory of Eskisehir Osmangazi University. Figure 2(a) displays the SEM image of the uncoated surface at 500x magnification, while Figure 2(b) displays the SEM photo of the Mo treated surface of the coil at 5000x magnification. Figure 2(b) shows that the surface morphology of the coil changes due to the coating, resulting in an increase in surface roughness. Although the roughness is not evenly distributed on the surface of the coil, it effectively trapped the analyte atoms. The EDS data of the Mo-coated W-coil is illustrated in Figure 3. This result proves that the coil surface is successfully coated with Mo particles.

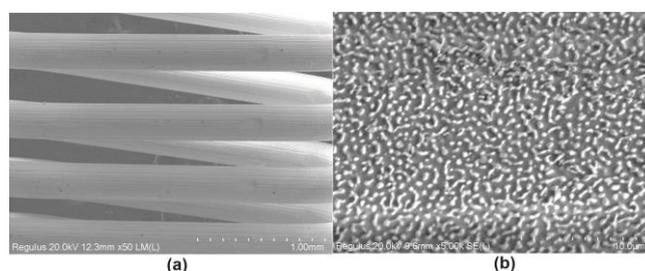


Figure 2. SEM pictures of the (a) bare W-coil at magnification of 50x (b) the Mo-coated W-coil at magnification of 5000x.

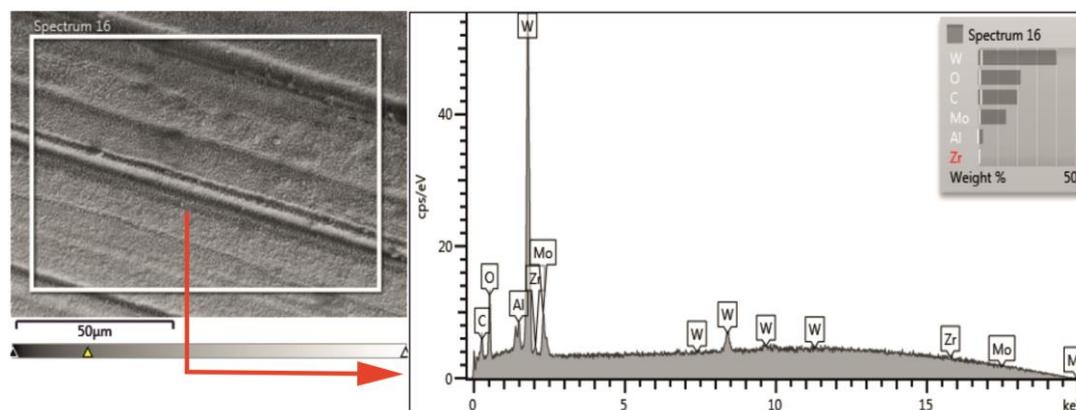


Figure 3. The outcome of EDS analysis of the Mo-coated W coil.

### 3.3. Figures of Merit

The analytical performance data for the proposed technique and the traditional CV-AAS method were determined utilizing the optimal operating settings described above, and the findings are given in Table 2. Using only 120 s of collection time with the developed technique, a 35.6-fold increase in limit of detection (LOD) was achieved compared to the conventional method. Comparing the  $C_0$  values, the sensitivity increase was 39.7, showing a significant difference between the traditional CV-AAS approach ( $0.278 \mu\text{g L}^{-1}$ ) and the advanced technique ( $0.007 \mu\text{g L}^{-1}$ ).

Table 2. Figures of the merit of the proposed technique and the traditional technique.

	Proposed technique	CV-AAS technique
Limit of detection, $\mu\text{g L}^{-1}$	0.017	0.605
Limit of quantification, $\mu\text{g L}^{-1}$	0.573	2.017
Relative standard deviation, %	3.6	2.8
Characteristic concentration, $\mu\text{g L}^{-1}$	0.007	0.278
Linear range, $\mu\text{g L}^{-1}$	0.1-10.0	8.0-40.0
Analyte volume, mL	10.7	-
Collection time, s	120	-

A comparison of the LOD value achieved in this study with that of some other techniques used to determine mercury concentration in the literature is given in Table 3. It can be posited that the LOD value obtained in this study is competitive with previously published plasma-derived methods and similar trapping studies.

Table 3. Comparison of the LOD of the proposed technique with that of previously published studies.

Technique	LOD ( $\mu\text{g L}^{-1}$ )	Reference
ICP-MS	0.010	[23]
ICP-OES	0.02	[24]
CVG-AFS	0.33	[25]
Silver trap -FI-CVAAS	0.003	[26]
W-coil-trap FI-VGAAS	0.6	[27]
Au NP coated-FI-CVG-AAS	0.01	[28]
Au-coated W-coil trap CV-AAS	0.006	[8]
Mo-coated W-coil CV-AAS	0.017	This study

### 3.4. Accuracy check

To verify the accuracy of the developed setup, the Hg content of NIST SRM 1641e and dolt:5 were analyzed using the optimal parameters given in the section above.

The values for Hg in the NIST SRM 1641e and dolt:5 were determined to be  $0.1028 \pm 0.006 \text{ mg L}^{-1}$  and  $0.47 \pm 0.11 \text{ mg kg}^{-1}$ , respectively. The certified values for these samples were  $0.1016 \pm 0.0017 \text{ mg L}^{-1}$  and  $0.44 \pm 0.18 \text{ mg kg}^{-1}$ . The outcomes closely matched the certifying values at a 95% probability level.

### 3.5. Investigation of the interference effects of hydride forming elements

In order to examine the impact of some hydride-generating elements (As, Sb, Sn, and Se) on T-Hg signal, interference studies were conducted. To this end, three distinct solutions were prepared, maintaining a constant mercury concentration of  $2.0 \mu\text{g L}^{-1}$  while varying the concentrations of interferences at 1, 10, and 100 times the analyte concentration. Under ideal operational settings, the experiments were executed with a trapping duration of 120 s, and the results are presented in Table 4. Hydride-forming ions were observed to enhance the analyte signal to a slight extent, although no significant interference effects were noted at the concentrations studied.

Table 4. The impacts of some hydride-generating elements on analyte signal.

Element	The percentage of recoveries in the presence of an interferent concentration ( $\mu\text{g L}^{-1}$ )		
	1.0	10.0	100.0
As	$98.5 \pm 1.3$	$100.9 \pm 1.8$	$107.6 \pm 2.1$
Sb	$101.8 \pm 1.5$	$103.6 \pm 2.4$	$110.2 \pm 1.6$
Sn	$99.6 \pm 2.8$	$104.0 \pm 2.3$	$109.6 \pm 2.9$
Se	$97.3 \pm 2.2$	$99.5 \pm 1.8$	$104.9 \pm 2.7$

The mean value and corresponding standard deviation are presented as results ( $n = 3$ )

### 3.6. Analysis of real samples

Tap water and fish muscle samples were analyzed utilizing the proposed methodology. The results of water samples indicated that the concentration of T-Hg was below the analysis limits. The standard addition method was used by adding known concentrations of 2.0 and  $2.5 \mu\text{g L}^{-1}$  Hg to the tap water samples. The findings are given in Table 5. The T-Hg concentrations in fish muscle tissue samples are also presented in Table 6.

Table 5. Measurement of T-Hg in spiked water samples.

Sample	Initial reading ( $\mu\text{g L}^{-1}$ )	Spike ( $\mu\text{g L}^{-1}$ )	Last reading ( $\mu\text{g L}^{-1}$ )	Recovery (%)
Tap Water 1	<LOD	2.00	2.23±0.09	111.3
Tap Water 2	<LOD	2.00	2.11±0.05	105.5
Tap Water 3	<LOD	2.50	2.66±0.10	106.6
Tap Water 4	<LOD	2.50	2.62±0.08	104.9

The mean value and corresponding standard deviation are presented as results (n = 3)

Table 6. Measurement of T-Hg in fish muscle tissue samples.

Sample	T-Hg concentration ( $\mu\text{g kg}^{-1}$ )
Sea bass 1	68.3 ± 4.5
Sea bass 2	51.8 ± 4.9
Sea bass 3	75.2 ± 3.6
White seabream 1	32.8 ± 2.5
White seabream 2	39.7 ± 3.9
White seabream 3	28.2 ± 3.3

The mean value and corresponding standard deviation are presented as results (n = 3)

#### 4. Conclusion

This study introduces the combination of a Mo-coated W-coil with the CV-AAS for ultra-trace determination of Hg in tap water and fish muscle tissue samples, marking the first application of this technique. The  $C_0$  value of the developed technique exhibited a 39.7-fold improvement in sensitivity in comparison to the CV-AAS method. Moreover, the LOD of the advanced technique was comparable to highly sensitive techniques like ICP-MS and ICP-OES. The applicability of the methodology was demonstrated by analyzing Hg concentrations in the CRMs and several tap water samples, with recoveries ranging from 104.9% to 111.3% for tap water samples. The concentration of Hg in sea bass and white sea bream samples ranged from 51.8 to 75.2  $\mu\text{g kg}^{-1}$  and from 28.2 to 39.7  $\mu\text{g kg}^{-1}$ , respectively. These values are below the upper limits set by both the European Union and the United States. The cost-effectiveness of the instrument and atom trap stands out as a primary advantage of the developed technique. This technique presents an economical and practical option for analyzing Hg in environmental and food samples, particularly beneficial for researchers facing budget constraints and lacking access to high-cost devices like ICP-MS and ICP-OES.

#### 5. Acknowledgment

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