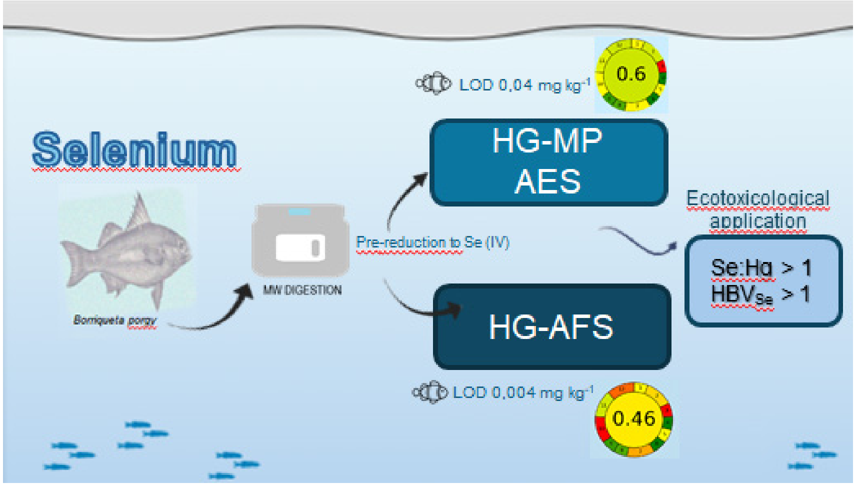
Brazilian Journal of Analytical Chemistry
Pre-publication – Accepted after peer review
doi: 10.30744/brjac.2179-3425.AR-58-2025

ARTICLE

Development of Methods for Selenium Determination in Fish: Tools for Ecotoxicological Studies

Lucía Falchi, Juan Gularte, Fiorella Iaquinta*

Grupo Química Analítica Ambiental, Facultad de Química, Universidad de la República
Av. Gral. Flores 2124, 11800 Montevideo, Uruguay



Selenium (Se) plays a significant role in many physiological processes. During the past years the role of Se has changed, from being considered toxic to the definition of being essential in almost every cell of our body. Furthermore, Se species play a role in mercury (Hg) detoxification suggesting that the protective effect of Se against Hg is related to the amount of Se available. In this work, two methods for Se determination in fish, by HG-AFS and HG-MP AES were developed. Moreover, a green analysis was applied to evaluate them. To both methods, optimization conditions were exhaustively evaluated and validated. Excellent figures of merit were obtained, with LOD of 0.04 mg kg^{-1} and 0.004 mg kg^{-1} to HG-MP AES and HG-AFS, respectively. The developed methods fit our purpose, being adequate for the determination of Se in fish and were compared in terms of accordance with the green analytical chemistry principles using AGREE metrics. The HG-MP AES method constitutes a greener alternative (0.60) than AFS (0.46). Borriqueta porgy (*Boridia grossidens*) fish samples from the Uruguayan coast were analyzed. The levels of Se in the samples were between 0.15 – 0.40 mg kg^{-1} determined by HG-MP AES and 0.13 – 0.35 mg kg^{-1} with HG-AFS, being the developed methods two alternatives for the Se monitoring. Finally, an ecotoxicological study was conducted to evaluate Se protection against Hg in fish tissue. All samples presented a Se:Hg molar ratio and the Selenium Health Benefit Value above 1, suggesting the protection of Se against mercury toxicity. This work presents two developed analytical methods suitable for the determination of Se in fish samples; in addition, this is the first evaluation that presents HG-MP AES for Se determination in fish. Furthermore, this work constitutes the first to determine Se in Uruguayan coast as well as the first to evaluate the Se protection against Hg in fish tissue.

Cite: Falchi, L.; Gularte, J.; Iaquinta, F. Development of Methods for Selenium Determination in Fish: Tools for Ecotoxicological Studies. *Braz. J. Anal. Chem.* (Forthcoming). http://dx.doi.org/10.30744/brjac.2179-3425.AR-58-2025

Submitted June 30, 2025; Resubmitted September 2, 2025; Accepted September 30, 2025; Available online October 2025.

This article was submitted to the BrJAC special issue on the 8th Uruguayan Congress of Analytical Chemistry (CUQA 8 2024).

Keywords: selenium, fish, HG-MP AES, HG-AFS, ecotoxicology

INTRODUCTION

Selenium (Se) exists in both inorganic and organic forms. In its inorganic forms, Se is commonly found as selenate and selenite. Its organic forms include selenomethionine and selenocysteine. Geochemically, Se is primarily found in crustal rocks and phosphate-rich soils, and is introduced into aquatic ecosystems through both natural sources and human activities. Anthropogenic activities, such as mining, coal combustion, oil refining, and wastewater from agricultural drainage, are the main sources of Se contamination in aquatic systems, leading to elevated concentrations that are toxic to aquatic organisms.[1]

Selenium is a vital micronutrient that helps maintain physiological homeostasis in all vertebrates. However, these elements can become toxic when present in supraphysiological concentrations. It can move through aquatic food chains via bioconcentration and biomagnification. Given that, fish are top predators in most aquatic ecosystems and provide approximately 60% of the total animal protein consumed by humans, for this reason Se accumulation poses a significant food safety concern.[1,2] However, it seems that Se species play a role in mercury (Hg) detoxification.[3-6]

Mercury is a well-known toxic metal that has the capability of bioaccumulation, bioconcentration and biomagnification. Its main toxic form, methylmercury (II), causes severe damage to the nervous system, increases in severity of nephrotoxicity and blood pressure, among other effects. One of the main sources of Hg intoxication is throw diet, mainly to fish or seafood.[7]

It is suggested that the protective effect of Se against Hg is related to the amount of Se available. This occurs when the Se concentration is higher than Hg in tissues, and the Hg is sequestrated. A parameter that evaluates the protection of Se against Hg is the molar concentration ratio, where tissues need to exceed.[3,4]

Due to its capacity of volatile generation species, Se is commonly determined by hydride generation using borohydride as a reductant.[8] The hydride generation could be coupled to atomic fluorescence spectrometry (HG-AFS) or atomic absorption spectrometry (HG-AAS).[9,10] HG-AFS continues to be a widely used technique due to its high sensitivity and outstanding limits of detection.[11] Additionally, the Se determination could be performed using inductively coupled plasma mass spectrometry (ICP-MS).[12-14] Also, Se determination could be carried out by hydride generation coupled to Microwave Plasma Atomic Emission Spectrometry (HG-MP AES). The MP AES uses plasma generated by nitrogen gas obtained from an air compressor and a nitrogen generator.[15] MP AES "runs on air", so the costs are reduced and the need for the supply of flammable or expensive gases is eliminated. Furthermore, ICP-OES limit of detection reported in complex matrix are in the same order of MP AES.[16,17] For this reason, it is a less expensive and more environmentally friendly offer than the ICP-OES.[15] The determination of Se by HG-MP AES has been developed for meat samples before.[16] However, to the best of our knowledge, this is the first work to determine Se in fish samples by HG-MP AES.

This work presents the development of two methods for the determination of Se in fish, by HG-AFS and HG-MP AES. A green analysis was applied to evaluate both methods. Then, fish samples were analyzed, as an application to ecotoxicological studies; Se protection against Hg was evaluated for the first time on the Uruguayan coast.

MATERIALS AND METHODS

Reagents

Standard solutions for calibration curves were prepared by serial dilution of commercial atomic absorption stock solutions (1000 mg L^{-1}) of Se and Hg (Merck, Darmstadt, Germany) in acidic media. Nitric acid (HNO_3) 70% w w^{-1} (Merck, Darmstadt, Germany) was used for microwave-assisted digestions. Ultrapure water (ASTM Type I) of 18.2 MΩ cm resistivity was obtained from a Millipore™ DirectQ3 UV water purification system (Bedford, Massachusetts, USA). Sodium tetrahydroborate ($NaBH_4$) solutions were prepared from the salt (Fluka, Hauppauge, USA) in 0.5% w v^{-1} NaOH (Merck, Darmstadt, Germany). Hydrochloric acid (HCl) (Merck, Darmstadt, Germany) was used to pre-reduce Se (VI) and as the carrier in the determination of Se employing AFS. All reagents were of analytical grade.

Samples

According to Muniz et al. (2004),[18] Montevideo harbour presented Hg levels that could be harmful to the ecosystem. For this reason, a representative coastal species of fish such as Borriqueta porgy (*Boridia grossidens*) was sampled from Montevideo, Uruguay harbour in winter from July to August 2024.

Fish samples were dissected using a stainless-steel knife, and muscle samples were crushed using a knife mill and preserved in polypropylene tubes at -4 ºC until analysis.

A certified reference material of Dogfish Liver (NRC-DOLT-5, National Research Council Canada, Canada) was used for the optimization and method validation.

Sample digestion

A microwave-assisted acid digestion was performed using CEM Mars6 microwave equipment (Matthews, NC, USA) provided with 12 Easy Prep Plus® Teflon vessels. For sample preparation, 0.25 g of sample was accurately weighed into each reaction vessel, and 10.0 mL of 4.8 mol L^{-1} HNO_3 was added. The program consisted of a 15-minute ramp to 200 °C, holding for 30 minutes, and then cooling to room temperature. Power varied between 400 and 1800 W, with a maximum pressure of 3.45 MPa. After mineralization, samples were filled up to 15.0 mL with ultrapure water. Samples and reagent blanks were run in duplicate. Reagent blanks were also run.

Sample pre-reduction

Since lower oxidation states can generate Se hydrides, firstly, it is necessary to reduce Se (VI) to Se (IV).[8] For this task, 5.0 mL of HCl 12 mol L^{-1} were added to 5.0 mL of the digested sample and heated at 100 °C for 50 minutes using a heating plate.[16] Calibration solutions were also run according to this procedure. Finally, the online reduction with $NaBH_4$ generates the selenium hydride (H_2Se).

Analytical determinations

Selenium determination by microwave-induced plasma optical emission spectrometry (MP AES) was performed with an Agilent 4210 spectrometer (Agilent Technologies, Santa Clara, USA). An online nitrogen generator, Agilent 4107 (Agilent Technologies, Santa Clara, USA), fed from environmental air through a KK70 TA-200 K compressor (Agilent Technologies, Santa Clara, USA) was used. The instrument was equipped with a multimode spray chamber (MSIS) for vapor generation and a standard torch (Agilent Technologies, Santa Clara, USA). The instrumental parameters are presented in Table I. Mercury determinations were also performed by MP AES coupled to cold vapor using $NaBH_4$ 2 % w v^{-1} as reductant according to Iaquinta et al. 2024.[19]

Table I. Instrumental parameters of HG-MP AES to determine Se

MP AES instrumental parameter	Optimized condition
Pump speed (rpm)	30 (0.90 mL min^{-1})
Nitrogen flow (L min^{-1})	1.0
Reading time (s)	10
Viewing position	-10
Stabilization time (s)	10
Wavelength (nm)	196.026
Background correction	Automatic

Meanwhile, Se determination by atomic fluorescence spectroscopy (AFS) was performed with a Persee PF7 Spectrophotometer (Persee Analytics, Beijing, China). Equipped with an integrated continuous flow hydride system, quartz atomization system, and high-intensity hollow cathode lamp (HCL) used as the radiation source (Persee Analytics, Beijing, China). Argon 99.998% (Praxair, Montevideo, Uruguay) was used as a gas carrier. Instrumental conditions were optimized, achieving the following parameters: argon flow of 200 L min^{-1}, sample injection of 1.5 mL, lamp main current of 40 mA, atomizer temperature of 200 °C, and 30 s for the lecture time.

Method validation

Validation was performed according to the recommendations of the Eurachem Guide.[20] The figures of merit evaluated were linear range, limit of detection (LOD), limit of quantification (LOQ), precision, and trueness. Precision and trueness were evaluated using dogfish liver CRMs.

RESULTS AND DISCUSSION

Optimization of HG-MP AES conditions

A previously validated method was used to determine Se in beef samples through HG-MP AES.[16] Based on these conditions, some parameters were optimized to fit our purpose. Pump speed, nitrogen flow, view position, and $NaBH_4$ concentration were evaluated.

Three different concentrations of $NaBH_4$ dissolved in NaOH 0.5% w v^{-1} were used to determine the optimal concentration to generate the hydride. For this task, certified reference material was used, and the evaluation was the percentage of signal recovery in duplicate. Table II presents the results and conditions evaluated. Since experiments 2 and 3 presented similar recoveries, it was decided to continue with $NaBH_4$ 2.0% w v^{-1} (Experiment 2). This selection was made to seek the lower amount of $NaBH_4$ to achieve a quantitative reduction of Se. Then, the analytical optimization was performed using this concentration.

Table II. Optimization results in using the HG-MP AES method

Experiment	$NaBH_4$ (% w v^{-1})	% Signal Recovery*
1	1.5	86 ± 1
2	2.0	100 ± 1
3	3.0	101 ± 1

*Signal recovery (%) = [intensity of CRM signal * 100] / [intensity of CRM signal obtained on calibration curve conditions (Experiment 2)].

Once the hydride conditions were optimized, the view position and the nebulizer flow were evaluated using a standard of 20 µg L^{-1}. The nebulizer flow optimization was evaluated between 0.30 and 1.00 L min^{-1}, obtaining the highest signal at 1.00 L min^{-1}. On the other hand, the viewing position was optimized, obtaining a result of -10, in a range from steps -120 to 120. Results are presented in Figure 1.

Finally, the pump speed was evaluated between 15 (0.45 mL min^{-1}) and 45 rpm (1,35 mL min^{-1}). The best condition was 30 rpm (0.9 mL min^{-1}). For this task, sensibility (slope of the calibration curve) was evaluated, resulting on highest sensibility at 30 rpm (0.9 mL min^{-1}) with a value of 18.4736 L µg^{-1}.

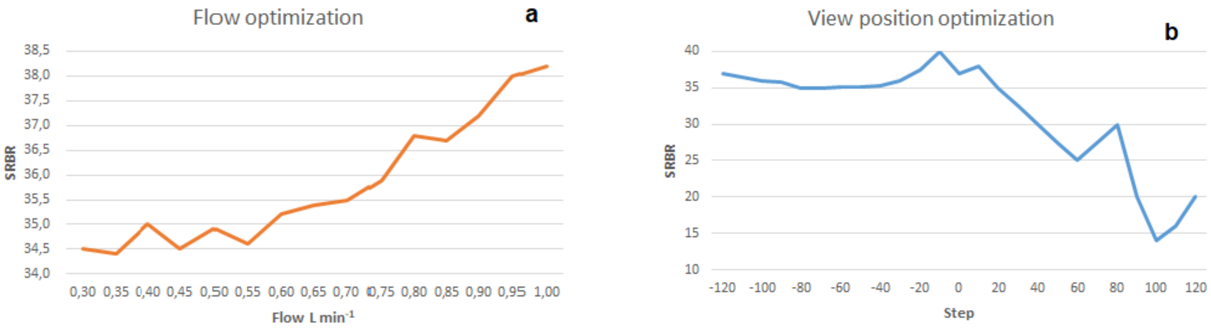


Figure 1. Flow (a) and view position (b) optimization for Se determination by HG-MP AES.

Optimization of HG-AFS conditions

For Se determination by HG–AFS, the recommended conditions of the Operational Persee Manual (Persee Analytics, Beijing, China) were considered. Based on them, some parameters were optimized to fit our requirements. The evaluated parameters were carrier and reductor ($NaBH_4$) concentrations, gas carrier flow, and sample injection volume, for the proper hydride generation.

A solution of HCl was employed as a carrier for Se determination. Three different acid concentrations (2.5, 5, and 7.5% v v^{-1}) were evaluated for the optimal hydride generation. Using the higher signal (area) for the dogfish liver CRM solution as a criterion, the optimal condition obtained was 5% v v^{-1}.

Under those carrier optimized conditions, the reductor concentration ($NaBH_4$), injection volume, and gas flow were evaluated using a composite-central experimental design of three variables in three levels.[21] The reductor concentrations studied were between 0.4 and 2.5% w v^{-1} in 0.5% w v^{-1} NaOH solution, the gas flow range was 200-400 mL min^{-1}, and the injection volume between 1.0 and 2.0 mL. All the experiments were done using the dogfish liver CRM solutions. The signals (area) obtained from the experimental design were analyzed in STATISTICA 8.0 software (StatSoft Inc, USA), gathering a graphic shown on Figure 2. Since 1.5 mL presents the highest area (550 IF), showing significant differences between 1 and 2 mL, the response surface was plotted with reductor concentration (Y), gas flow (X) and area (Z).

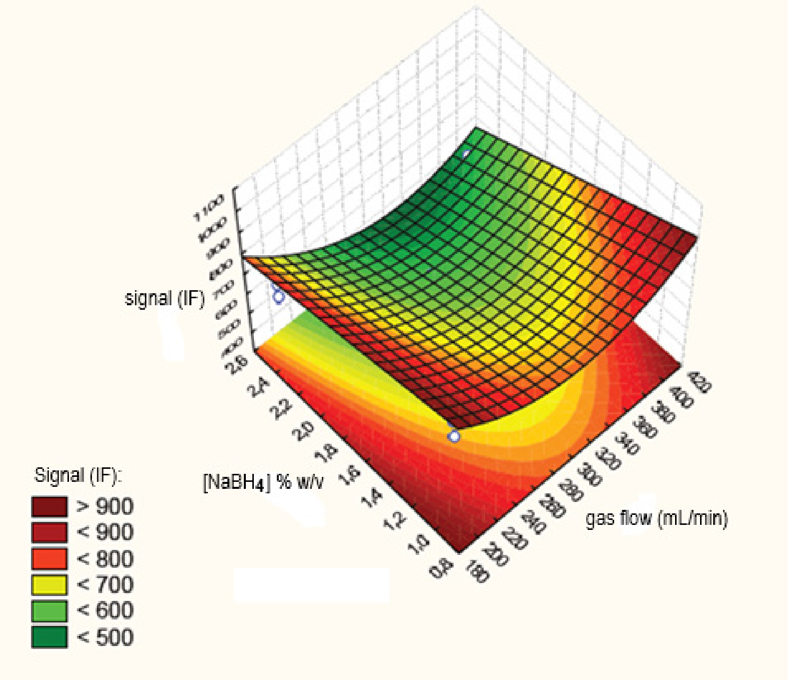


Figure 2. Results of the composite-central experimental design.

Finally, the optimal conditions for the proper hydride generation and detection of Se were under an HCl carrier concentration of 5% v v^{-1}, with an injection of 1.5 mL, flow gas of 200 mL min^{-1} and $NaBH_4$ reducer of 0.8% w v^{-1} in a 0.5% w v^{-1} NaOH solution.

Method validation

Once the critical parameters for hydride generation and the instrumental conditions were optimized, both methods were validated. The figures of merit evaluated, and their results are presented in Table III.

Table III. Performance parameters obtained for the proposed methods

Figure of merit	HG-MP AES	HG-AFS
Linear range (µg L^{-1}, n=6)	2.45 - 20.00	0.21 - 20.00
LOD (instrumental limits / method limits)	0.73 µg L^{-1} / 0.04 mg kg^{-1}	0.065 µg L^{-1} / 0.004 mg kg^{-1}
LOQ (instrumental limits / method limits)	2.45 µg L^{-1} / 0.15 mg kg^{-1}	0.210 µg L^{-1} / 0.013 mg kg^{-1}
Precision (RSD%, n=6)	5.8	3.8
Trueness (R%, n=6)	92 ± 5	86 ± 3
t-experimental	-2.54	1.68
t (0.05; 5)	2.57	

Linearity was evaluated by visual inspection and determinant coefficients after the evaluation of six points of calibration in triplicate. In all cases, the coefficient presented R^2>0.99, proper visual adjustment, and proper randomness study of the residuals. Figure 3 presents the calibration curve with the respective residuals, showing its randomness. Limits of detection and quantification were evaluated by 3*s*/b and 10*s*/b criteria. Where b is the slope of the calibration curve, and *s* is the standard deviation of 10 blanks previously pre-reduced. It is highlighted by the ten-times lower limits in the HG-AFS compared with HG-MP AES. HG-AFS limits allow the detection with reliability of lower levels of Se, being a strength of the technique. The obtained results are, in the same order or lower than the reported by recent works for HG-AFS.[22-24] Moreover, HG-AFS limits are in the same order as ICP-MS.[12-14] However, the results obtained with both methods were adequate for our purpose due to the Se content in fish samples previously reported in Atlantic Ocean.[5] Precision was estimated as repeatability and expressed as a percentage relative standard deviation (%RSD) after the analysis of the CRM (n=6). In both methods, it was below 6% which is suitable for our purpose since Horwitz theory about variability at trace levels has been proposed in 15% RSD.[25] For trueness evaluation, recoveries of the CRM were evaluated with results of 85-100%. Also, a student's *t*-test was performed to compare the obtained values with the certified values of the CRM. Table III presents the results obtained after the statistical analysis. Experimental *t*-values obtained were below the theoretical *t* (0.05, 5) = 2.57, indicating that at the 95% confidence level, the experimental concentrations did not differ significantly from the certified ones.[26]

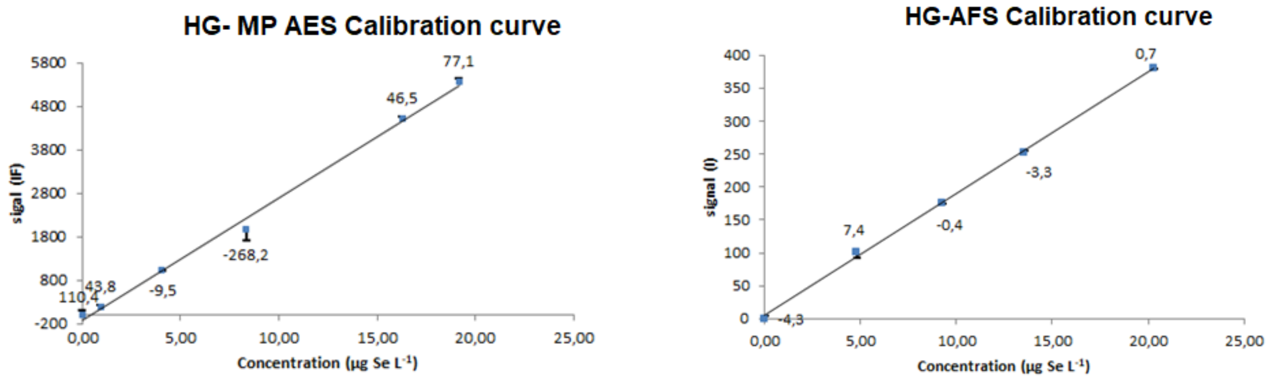


Figure 3. Calibration curve and residuals for Se determination by means of HG -MP AES (left) an HG-AFS (right).

The performance of the analysis between the developed methods was also evaluated by comparing the obtained Se levels in fish. For this task, a *t*-student test of mean values was applied. The result is presented in Table IV, where the means obtained by both methods were comparable since they did not differ significantly at a significance level of 95%.

Both methods fit our purpose, being adequate for the determination of Se in fish. It is worth noting that to the best of our knowledge, this is the first work that presents HG-MP AES for Se determination in fish.

Table IV. Comparison of methods by Student´s *t*-test

	HG-MP AES	HG-AFS
Average	0.303	0.254
t experimental	0.45	
t (0.05; 6)	2.23	

Greenness analysis

Both methods were compared in terms of accordance with the green analytical chemistry (GAC) principles.[27] There are many tools that allow the greenness assessment, Green Analytical Procedure Index (GAPI), AGREEPrep, Eco-scale.[28,29] For this task, the Analytical GREEnnes (AGREE) metric tool was applied.[26] This metric evaluates the 12 principles of green analytical chemistry individually, having each principle its type of evaluation. Finally, the result is a figure with colors and numbers, where the green color and the number 1 represent a complete approximation to green analytical chemistry principles.[30] Since sample preparation is the same for both methods, this tool provides more information than the AGREEPrep. Also, the AGREE pictograms are more friendly and easier to compare than GAPI pictograms or Eco-scale categories. Figure 4 presents the results of the AGREE evaluation. As can be observed, HG-MP AES method is more aligned with the green analytical chemistry principles, with a total score of 0.60. The main problems for both methods are that they are offline methods (Principle 3) and require a sample treatment (Principle 1). However, HG-MP AES presents advantages compared to the HG-AFS since it uses lower amounts of reagents, with lower toxicity and safer conditions, which is better for the environment and operator´s safety (Principles 2 and 12). Also, it generates a higher volume of waste (Principle 7). But the main difference is presented in principle 10, which involves renewable sources, and HG-MP AES uses air as a consumable, meanwhile HG-AFS uses argon. Thus, the HG-MP AES method constitutes a greener alternative than AFS.

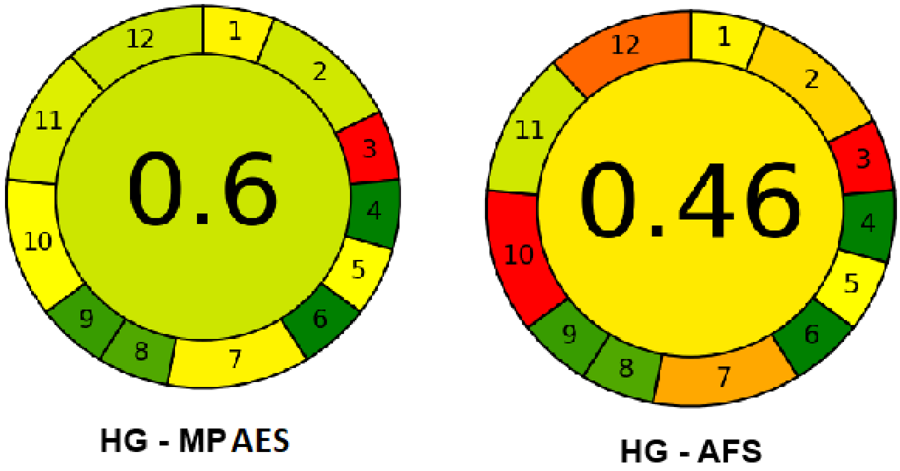


Figure 4. Results of AGREE metrics for the methods used.

Application to fish samples

Borriqueta porgy is a common fish from the southwest Atlantic Ocean, which is found in Uruguay, Brazil, and Argentina. However, there is no evidence of Se determination in fish of the Uruguayan coast. In this work, six fish samples were analyzed in duplicate using both methods for Se determination. The levels of Se in the samples were between 0.15 – 0.40 mg kg^{-1}, determined by HG-MP AES, and 0.13 – 0.35 mg kg^{-1} with HG-AFS.

The Se levels found in this work are in the same range as previous studies of fish in the Atlantic Ocean,[5] and also in the Mediterranean Sea.[31] Since fish is a good model for detecting toxic and nutritional chemicals in aquatic ecosystems,[32] the developed methods are two alternatives for the Se monitoring.

Selenium:mercury evaluation

In this work an ecotoxicological application was studied: the evaluation of Se protection against Hg. Selenium has an important role in the sequestration and reduction of bioavailability of methylmercury, suggesting its relevance in the reduction of mercury toxicity.[4,5] There are many studies in which the evaluation of the selenium:mercury molar ratio is presented in fish muscle. This ratio provides information about the protection against mercury toxicity. When the ratio is above 1 suggests that it is protecting against Hg.[4-6] Also, a Health Benefit Value (HBV) has been proposed as an indicator of sufficient selenium in fish in comparison to mercury. A positive Se-HBV suggests that Se could protect against Hg toxicity.[4,33]

The selenium:mercury (Se:Hg) molar ratio was evaluated using Equation 1:[6]

$$Se{:}Hg = \frac{Se\ concentration\left(\frac{g}{kg}\right)\Big/78.9}{Hg\ concentration\left(\frac{g}{kg}\right)\Big/200.59} \qquad \text{Equation 1}$$

Meanwhile, Selenium Health Benefit Value was evaluated using Equation 2:[33]

$$HBV\ (Se) = \frac{(Se - Hg)}{Se\ \times\ (Se + Hg)} \qquad \text{Equation 2}$$

Table V presents the results for the six samples evaluated.

Table V. Total selenium and mercury content, molar ratios, and HBV_{Se}

Selenium (mg kg^{-1})	Mercury (mg kg^{-1})	Se:Hg ratio	HBV_{Se}
0.345 ± 0.011	0.108 ± 0.017	8.14	4.31
0.256 ± 0.021	0.088 ± 0.013	7.41	3.19
0.250 ± 0.020	0.211 ± 0.087	3.01	2.81
0.294 ± 0.027	0.251 ± 0.019	2.98	3.31
0.197 ± 0.044	0.162 ± 0.022	3.09	2.23
0.305 ± 0.016	0.108 ± 0.014	7.18	3.79

Mean concentration ± standard deviation.

As we can observe, all samples presented a Se:Hg molar ratio above 1, suggesting the protection of Se against the mercury toxicity. As we can notice, even when the samples are from the same fish species, the ratio may vary; this could be related to the few samples evaluated. Nevertheless, this constitutes the first work that evaluates this relationship in Uruguayan samples. Also, the HBV_{Se} is positive in all cases, reinforcing the Se protection in the evaluated samples.

CONCLUSIONS

Two analytical methods were developed and validated for the determination of Se in fish muscle tissue. Both methods were adequate for the purpose proposed, HG-MP AES being more aligned with the Green Analytical Chemistry principles. To the best of our knowledge, this constitutes the first work assessing Se in fish through HG-MP AES.

The methods proposed were used for the determination of Se in Borriqueta porgy samples from Montevideo, Uruguay. An ecotoxicological application for a preliminary evaluation of Se protection against mercury was conducted in each sample, showing that it has a protective role in fish samples from Montevideo bay. However, more samples should be analyzed to confirm this protective activity.

Finally, these methods, HG-AFS and HG-MP AES could be used for the Se determination in ecotoxicological studies.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

The authors thank “Agencia Nacional de Investigación e Innovación” (ANII) and the “Programa de Desarrollo de las Ciencias Básicas” PEDECIBA-Química.

REFERENCES

(1) Uddin, M. H.; Ritu, J. R.; Putnala, S. K.; Rachamalla, M.; Chivers, D. P.; Niyogi, S. Selenium Toxicity in Fishes: A Current Perspective. *Chemosphere* **2024**, *364*, 143214. https://doi.org/10.1016/j.chemosphere.2024.143214

(2) Tóth, R. J.; Csapó, J. The Role of Selenium in Nutrition – A Review. *Acta Universitatis Sapientiae, Alimentaria* **2018**, *11* (1), 128–144. https://doi.org/10.2478/ausal-2018-0008

(3) Ribeiro, M.; Zephyr, N.; Silva, J. A. L.; Danion, M.; Guérin, T.; Castanheira, I.; Leufroy, A.; Jitaru, P. Assessment of the Mercury-Selenium Antagonism in Rainbow Trout Fish. *Chemosphere* **2022**, *286*, 131749. https://doi.org/10.1016/j.chemosphere.2021.131749

(4) Looi, L. J.; Aris, A. Z.; Haris, H.; Yusoff, F. M.; Hashim, Z. The Levels of Mercury, Methylmercury and Selenium and the Selenium Health Benefit Value in Grey-Eel Catfish (*Plotosus canius*) and Giant Mudskipper (*Periophthalmodon schlosseri*) from the Strait of Malacca. *Chemosphere* **2016**, *152*, 265–273. https://doi.org/10.1016/j.chemosphere.2016.02.126

(5) Burger, J.; Gochfeld, M. Mercury and Selenium Levels in 19 Species of Saltwater Fish from New Jersey as a Function of Species, Size, and Season. *Sci. Total Environ.* **2011**, *409* (8), 1418–1429. https://doi.org/10.1016/j.scitotenv.2010.12.034

(6) Burger, J.; Jeitner, C.; Donio, M.; Pittfield, T.; Gochfeld, M. Mercury and Selenium Levels, and Selenium:Mercury Molar Ratios of Brain, Muscle and Other Tissues in Bluefish (*Pomatomus saltatrix*) from New Jersey, USA. *Sci. Total Environ.* **2013**, *443*, 278–286. https://doi.org/10.1016/j.scitotenv.2012.10.040

(7) Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Mercury,* 1999. https://www.atsdr.cdc.gov/toxprofiles/tp46.pdf (accessed 2025-02-04).

(8) Dedina, J.; Tsalev, D. L. *Hydride Generation Atomic Absorption Spectrometry*. Wiley, 1995.

(9) Cabañero, A. I.; Carvalho, C.; Madrid, Y.; Batoréu, C.; Cámara, C. Quantification and Speciation of Mercury and Selenium in Fish Samples of High Consumption in Spain and Portugal. *Biol. Trace Elem. Res.* **2005**, *103* (1), 017–036. https://doi.org/10.1385/BTER:103:1:017

(10) Robinson, J.; Shroff, J. Observations on the Levels of Total Mercury (Hg) and Selenium (Se) in Species Common to the Artisanal Fisheries of Seychelles. *Neurotoxicology* **2020**, *81*, 277–281. https://doi.org/10.1016/j.neuro.2020.09.017

(11) Zou, X.; Shen, K.; Wang, C.; Wang, J. Molecular Recognition and Quantitative Analysis of Free and Combinative Selenium Speciation in Selenium-Enriched Millets Using HPLC-ESI-MS/MS. *J. Food Compos. Anal.* **2022**, *106*, 104333. https://doi.org/10.1016/j.jfca.2021.104333

(12) Ohki, A.; Nakajima, T.; Hirakawa, S.; Hayashi, K.; Takanashi, H. A Simple Method of the Recovery of Selenium from Food Samples for the Determination by ICP-MS. *Microchem. J.* **2016**, *124*, 693–698. https://doi.org/10.1016/j.microc.2015.10.012

(13) Grotto, D.; Batista, B. L.; Carneiro, M. F. H.; Barbosa Jr., F. Evaluation by ICP-MS of Essential, Nonessential and Toxic Elements in Brazilian Fish and Seafood Samples. *Food Nutr. Sci.* **2012**, *03* (09), 1252–1260. https://doi.org/10.4236/fns.2012.39165

(14) Okati, N.; Moghadam, M. S.; Einollahipeer, F. Mercury, Arsenic and Selenium Concentrations in Marine Fish Species from the Oman Sea, Iran, and Health Risk Assessment. *Toxicol. Environ. Health Sci.* **2021**, *13* (1), 25–36. https://doi.org/10.1007/s13530-020-00062-6

(15) Agilent Technologies. *Microwave Plasma Atomic Emission Spectroscopy (MP-AES). Application eHandbook*. https://www.agilent.com/ (accessed 2025-06-22).

(16) Pistón, M.; Silva, J.; Suárez, A.; Belluzzi, M.; Iaquinta, F.; Panizzolo, L.; Méndez, C.; Cerminara, M. Estudio Del Contenido de Selenio En Carne Vacuna Luego Del Proceso de Cocción. *INNOTEC* **2020***, 19*. https://doi.org/10.26461/19.05

(17) Khan, N.; Jeong, I. S.; Hwang, I. M.; Kim, J. S.; Choi, S. H.; Nho, E. Y.; Choi, J. Y.; Kwak, B.-M.; Ahn, J.-H.; Yoon, T.; Kim, K. S. Method validation for simultaneous determination of chromium, molybdenum and selenium in infant formulas by ICP-OES and ICP-MS. *Food Chem.* **2013***, 141* (4)*,* 3566–3570. https://doi.org/10.1016/j.foodchem.2013.06.034

(18) Muniz, P.; Danulat, E.; Yannicelli, B.; Garcıa-Alonso, J.; Medina, G.; Bicego, M. C. Assessment of contamination by heavy metals and petroleum hydrocarbons in sediments of Montevideo Harbour (Uruguay). *Environ. Int.* **2004***, 29* (8)*,* 1019–1028. https://doi.org/10.1016/S0160-4120(03)00096-5

(19) Iaquinta, F.; Mollo, A.; Machado, I.; Vögler, R.; Banhos, S. G.; Nogueira, A. R. A. Microwave-induced plasma optical emission spectrometry coupled to vapor generation (VG-MIP OES) as an alternative technique for As and Hg determination for seafood surveillance. *Spectrochim. Acta, Part B* **2025**, *232*, 107281. https://doi.org/10.1016/j.sab.2025.107281

(20) Magnusson, B.; Örnemark, U. *Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics*, 2nd Ed., Eurachem, 2014.

(21) Massart, D.; Vandeginste, B.; Buydens, L.; De Jong, S.; Lewi, P.; Smeyers-Verbeke J. *Handbook of Chemometrics and Qualimetrics: Part A, Data Handling*, 1997.

(22) Schmitz, C.; Grambusch, I. M.; Lehn, D. N.; Hoehne, L.; de Souza, C. F. V. A Systematic Review and Meta-Analysis of Validated Analytical Techniques for the Determination of Total Selenium in Foods and Beverages. *Food Chem.* **2023**, *429*, 136974. https://doi.org/10.1016/j.foodchem.2023.136974

(23) LeBlanc, K. L.; Kumkrong, P.; Mercier, P. H. J.; Mester, Z. Selenium Analysis in Waters. Part 2: Speciation Methods. *Sci. Total Environ.* **2018**, *640–641*, 1635–1651. https://doi.org/10.1016/j.scitotenv.2018.05.394

(24) Skok, A.; Manousi, N.; Anthemidis, A.; Bazel, Y. Automated Systems with Fluorescence Detection for Metal Determination: A Review. *Molecules* **2024**, *29* (23), 5720. https://doi.org/10.3390/molecules29235720

(25) Horwitz, W.; Albert, R. The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision. *J. AOAC Int.* **2006**, *89* (4), 1095–1109. https://doi.org/10.1093/jaoac/89.4.1095

(26) Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry* (6th Ed.). Prentice Hall, Harlow, 2010, Vol. 2010.

(27) Gałuszka, A.; Migaszewski, Z.; Namieśnik, J. The 12 Principles of Green Analytical Chemistry and the SIGNIFICANCE Mnemonic of Green Analytical Practices. *TrAC, Trends Anal. Chem.* **2013**, *50*, 78–84. https://doi.org/10.1016/j.trac.2013.04.010

(28) Nowak, P. M.; Wietecha-Posłuszny, R.; Pawliszyn, J. White Analytical Chemistry: An approach to reconcile the principles of Green Analytical Chemistry and functionality. *TrAC, Trends Anal. Chem.* **2021**, *138,* 116223. https://doi.org/10.1016/j.trac.2021.116223

(29) Falchi, L.; Pistón, M.; Casarotto, G.; Carro, S.; Cajarville, C.; Iaquinta, F. Development of a Green Methodology for the Determination of Artisanal Danbo Cheese Quality Parameters. *Braz. J. Anal. Chem*. **2023**, *10* (41), 30-41. https://doi.org/10.30744/brjac.2179-3425.AR-21-2023

(30) Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. AGREE—Analytical GREEnness Metric Approach and Software. *Anal. Chem.* **2020**, *92* (14), 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887

(31) Barone, G.; Storelli, A.; Meleleo, D.; Dambrosio, A.; Garofalo, R.; Busco, A.; Storelli, M. M. Levels of Mercury, Methylmercury and Selenium in Fish: Insights into Children Food Safety. *Toxics* **2021**, *9* (2), 39. https://doi.org/10.3390/toxics9020039

(32) Sumana, S. L.; Chen, H.; Shui, Y.; Zhang, C.; Yu, F.; Zhu, J.; Su, S. Effect of Dietary Selenium on the Growth and Immune Systems of Fish. *Animals* **2023**, *13* (18), 2978. https://doi.org/10.3390/ani13182978

(33) Ralston, N.; Raymond, L. Selenium Status and Intake Influences Mercury Exposure Risk Assessments. In: Banuelos, G. S.; Yin, X.; Lin, Z-Q. (Eds.). *Selenium in the Environment and Human Health*. Taylor & Francis Group, London, 2014.