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Assessment of selenium content in tropical fish species using hydride generation atomic absorption spectrometry

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Abstract

The present work aimed to investigate the chemical sides in hydride generation atomic absorption spectrometry (HG-AAS) (*i.e.*, a simple, low-cost, and sensitive approach) for selenium (Se) analysis in fish samples, and to assess the Se content in different tropical fish species. The limits of detection and quantification were of 0.25 and 0.75 µg/L, respectively, which were comparable to other similar methods employing HG-AAS. Good linearity (R^2 = 0.9999) was achieved within Se concentrations (0.50 to 10.0 µg/L). Favourable repeatability (RSD_r = 1.9%) and reproducibility (RSD_R = 3.5%) were obtained. DORM-4, a certified reference material, was used to evaluate the accuracy of the analytical method, and there was no statistically significant difference between the certified and measured values at the confidence level of 95%. For 24 collected samples of tropical fish species, the Se contents in marine fish were generally higher than those in freshwater fish (1,131.2 – 2,109.5 *vs.* 119.7 – 472.1 µg/kg) with high recoveries obtained from all spiked samples (95.1 to 99.1%).

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Introduction

Selenium (Se) is a trace metalloid, considered an essential nutrient for animals and human health, but only in small amount (Squadrone *et al.*, 2016). Se is essential for synthesising 25 selenoproteins, *e.g.*, glutathione peroxidase, thioredoxin reductase, and idothyronine deiodinases. The deficiency of Se can lead to the risks of heart disease, hypothyroidism, and a weak immune system. On the other hand, Se can be toxic, and may poison animals and humans when their intake exceeds a certain safety level (Choi *et al.*, 2009; Zhang *et al.*, 2020). Se intake of > 5 mg/day potentially causes hair loss, changes in nail morphology, diarrhoea, central nervous system disorders, kidney and liver damage, and loss of appetite (Choi *et al.*, 2009; Atasoy and Kula, 2022). Moreover, adequate Se intake levels can vary due to the differences in personal, physiological, and biological parameters (*e.g.*, body weight, age, and gender, *etc.*). Therefore, it is advised that the highest Se content in the dry diet should not exceed 2.0 mg/kg to balance its intake (Bakırdere *et al.*, 2018).

In the environment, Se is found in two main sources including natural (volcanic activity, decomposition of soil, rock, and sediment, *etc.*) and anthropogenic (industrial production, agriculture, medicine, waste incineration, *etc.*) sources. The inorganic forms of Se (*i.e.*, selenite and selenate) are primarily found in environmental samples, while the organic forms (*e.g.*, different methylated selenium species) are produced from wastewater, mud, and rock, and discovered in natural water and in biological systems. Fish and other seafood are major sources of Se in the human diet (Yang *et al.*, 2021). In their tissues, Se basically exists in the forms of selenocysteine, selenomethionine, selenium glutathione, and selenoproteins (Hariharan and Dharmaraj, 2020). It is known that fish can accumulate Se in various concentrations in their bodies due to the changes and differences in water sources, living depths, feeds, sizes, process of bioaccumulation, and excretion of fish in aquatic environment. Generally, inorganic Se usually found in natural water sources exhibit more toxicity than organic ones, and selenite-Se(IV) is more toxic than

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selenate-Se(VI) (Meche *et al.*, 2010; Qin *et al.*, 2015; Azad *et al.*, 2019; Atasoy and Kula, 2022).

To determine Se content in both biological and environmental samples, due to the severely low Se concentrations as well as other difficulties, *e.g.*, multiple oxidation states, matrix effects, and possible evaporation during sample preparation, *etc*., different procedures have been developed to preconcentrate the selenium concentration before analysis (Mendil *et al*., 2017; Panhwar *et al*., 2017; Acikkapi *et al*., 2019; Ali *et al*., 2021; Altunay and Tuzen, 2021; Atasoy and Kula, 2022). Hydride generation atomic absorption spectrometry (HG-AAS), graphite furnace atomic absorption spectrometry (HG-AAS), electrothermal atomic absorption spectrometry (ET-AAS), and inductively coupled plasma mass spectrometry (ICP-MS) are commonly employed as the analytical methods of Se after being digested by acid reagents (*e.g.*, nitric, sulphuric, perchloric acids, or a combination of them) in an open or a closed system (*e.g.*, microwave-assisted acid digestion) (Meche *et al.*, 2010; Sun *et al.*, 2013; Le *et al.*, 2013; 2021; Squadrone *et al.*, 2016; Bakırdere *et al.*, 2018; Nhon-Duc *et al.*, 2021; Atasoy and Kula, 2022). For measurement techniques, ICP-MS has many advantages including low detection limits, minimising chemical interferences, wide linear ranges, rapid analysis time, and capacities of isotope analysis. However, the ICP-MS alone or coupled with liquid chromatography (LC-ICP-MS) requires high equipment, operation, and maintenance cost. The AAS instruments are generally equipped in most analytical laboratories, and when the nebuliser is replaced by a hydride generation unit, the HG-AAS can be used to replace the ICP-MS as an easily operating, low-cost, and favourably sensitive detection method for Se at trace concentrations. In the HG-AAS approach, the formation of hydride species from Se is required. Before the hydride formation reactions, it is necessary to convert Se(VI) to Se(IV). There are different reducing agents such as TiC_3, KI, HCl, HCl and HBr, thiourea, and a mixture of KBr and HCl (Díaz-Alarcón *et al.*, 1996; Bohrer *et al.*, 2007; Le *et al.*, 2013; Atasoy and Kula, 2022). In several situations, HCl was used alone as a carrier solution because HCl could convert Se(VI) to Se(IV) without any additional reducing agent (Atasoy and Kula, 2022).

Based on available literature, most publications have focused on the preconcentration techniques to improve the analytical method performance, typically the sensitivity and limit of detection. Although the HG-AAS has been well developed and reported for many years, the chemical side, *e.g.*, the effects of carrier solution concentrations, the stability of reduced Se(IV), *etc*. have not been well discussed. This indicates a gap in information for researchers and analysts to ensure the result accuracy in the context of a high number of sample throughput, especially for routine analyses. Therefore, the present work was focused at evaluating the effects of carrier solutions of HCl during the hydride generation period, and the stability of Se(IV) after the reduction step. Before the HG-AAS analysis, acid digestion in an open system was employed to obtain the sample liquid. Then, the analytical method was applied to determine and assess the total Se contents in different kinds of tropical fish collected in Vietnam. The present work is among the very few publications that contribute to the scientific data of Se contents in Vietnamese fish samples, especially freshwater fish with their local features.

Materials and methods

Reagents and materials

Ultra-pure water (UPW) obtained from Millipore ultrapure water purification system (Millipore, Bedford, USA) was used throughout the experiment for solution preparation and dilution. The reducing reagent of 0.2% w/v $NaBH_4$ (Merck, Germany) was daily prepared in 0.05% w/v NaOH solution (Merck, Germany). HCl solutions at different concentrations (1.0, 1.5. 2.0, 2.5, 3.0, 4.0, 4.5, and 6.0 M) were obtained by the appropriate dilution of the concentrated commercial HCl (approximately 12 M; Merck, Germany) by UPW. The 1,000 mg/L commercial standard solution of Se (Merck, Germany) was used to prepare the immediate standard solutions of 50.0 mg/L in UPW, and 1.0 mg/L and 50.0 µg/L in 3 M HCl solution. The working solutions of 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.0 µg/L were prepared by diluting the 50.0 µg/L in 3 M HCl solution. All the containers and vessels were washed with 10% v/v nitric acid (Merck, Germany), then rinsed with UPW before use to avoid the risk of contamination. The standard solution of 6.00 µg/L was used for quality control (QC) to check the robustness of the instrument for each batch of six measurements.

The quartz T-shaped tube was utilised as the atomisation unit and pre-conditioned with a 1.0 mg/L

of Se solution to saturate its surface. The lengths of the horizontal and vertical arms of the T-shaped tube were 140 and 100 mm, respectively. For the horizontal arm, the inner and outer diameters were 15 and 18 mm, respectively. Additionally, the inner and outer diameters for the vertical arm were 6 and 9 mm, respectively. In case a remarkable decrease in the sensitivity was observed, the quartz tube was let immersed in a 5% v/v HF solution (Merck, Germany) for nearly 20 min to remove any possible contamination. Then, the tube was washed with UPW, and dried under a gentle stream of argon gas (99.95%) or in ambient conditions. After that, prior to reuse, the quartz tube was pre-conditioned with 1.0 mg/L of Se solution.

Sample collection and pre-treatment

A total of 24 commercial fish samples were purchased from producers and supermarkets around Ho Chi Minh City, Vietnam and belonged to two main types, including frozen marine fish and freshwater fish (Table 1). After being collected and transported to the laboratory, the fish samples were washed by UPW, and underwent the pre-treatment procedure as performed in TCVN 11489 (Vietnam National Standard, 2016). For farmed fish, they were skinned and shaved to get their tissue part by a plastic knife. All the fish samples underwent freeze-drying period within 48 h before being ground by ceramic pestle and mortar. Finally, the samples were kept in plastic polyethylene bags with zippers, and stored in a freezer until further analysis.

Table 1. Details on fish samples.

Type	Name	Length (cm)	Weight (kg)	Food source	Other notes
Marine fish	Tuna	50 - 200*	-	-	-
	Butterfish	30*	-	-	-
	Swordfish	430*	-	-	-
Freshwater fish	Snakehead fish	40**	~1.0	Insects, small shrimps, tiny fish	Ages: over 12 months
	Mullet fish	33**	0.4	Commercial fish feeding, zooplankton, suspended organic matters, bottom vegetation	Ages: 8 months, farmed
	Anabas	30**	0.2	Molluscs, tiny fish, plant, organic matters	Habitat: paddy fields, affected by fertilisers, pesticides, herbicides, *etc*.
	Catfish	40**	0.8	Insects, small snails, shrimps, tiny fish	Habitat: the lower mud layer of rivers
	Basa fish	40**	~1.0	Commercial fish feeding, snails, insects, plants	Ages: 8 months, farmed

*Provided by the fish suppliers; **Longest length from head to tail measured at the laboratory.

Measurement of selenium by HG-AAS

The atomic absorption spectrometer (Perkin-Elmer 603) equipped with a batch-mode hydride generation system (HG-AAS) was used for the measurement. Background correction mode using a deuterium (D_2) lamp was applied in the detection step. A hollow cathode lamp (HCL) was used as a light source. The lamp current, spectral bandwidth, and measurement wavelength were set to be 10.0 mA, 0.4 nm, and 196 nm, respectively. The operation temperature of the quartz tube during the atomisation period to break the bonds in the Se hydride species to form free Se atoms was 900 ± 4°C (pre-heated for at least 1 h before the measurement). Argon was utilised as the carrier gas (25 - 30 mL/min) to purge the generated hydride stream to the quartz tube. An argon gas flow meter was used to control its flow rate.

Sample preparation for selenium determination in fish

For the measurement of Se in fish, the samples were digested to obtain the liquids, then Se(VI) was reduced to Se(IV) prior to the generation of Se hydride species and atomisation. Acid digestion on an

open system was employed as the sample preparation procedure. Briefly, from 0.2 to 1 (± 0.001) g of well-homogenised fish sample was weighed and transferred into a 100-mL Erlenmeyer flask. Then, 5.0 mL of concentrated nitric acid (conc. HNO_3) was added. The mixture was heated on an electric stove equipped with a temperature control unit until the produced brown smoke subsided. The solution was cooled down to around 80 - 90°C, and further added with 2.0 mL of conc. HNO_3 and 1.0 mL of conc. $HClO_4$. Next, the heat was adjusted to increase slowly until the brown colour of NO_2 started to appear. The temperature was maintained to observe the white smoke appearance, and the stove was turned off. The solution was continued to cool down, added with 1.0 mL of conc. H_2SO_4, and heated gently and slowly. The sample preparation was stopped when the sample solution was clear and about 0.5 - 1.0 mL left.

Then, the sample solution from the flask was transferred into a 40-mL glass reaction tube with a cap to proceed with the reduction steps. The flask was rinsed at least three times using 15.0 mL of 6.0 M HCl solution, and all the rinsed solutions were collected into the reaction tube. After that, another 10.0 mL of 6.0 M HCl solution was further added to the tube (approximately 25.0 mL in total) to carry out the reduction period in a water bath at 90°C within 60 min. Finally, the reaction solution was cooled down to ambient temperature, transferred to a 50-mL volumetric flask, and made up to the calibration mark by UPW to be ready for the hydride generation and measurement steps.

For Se(VI) working standard solutions, the reducing period was carried out in a water bath at 90°C for 60 min using 25.0 mL of 6.0 M HCl as the reducing agent. Similar to the sample solutions, the liquid was finally made up to 50 mL by UPW.

The concentration of HCl in the sample solutions and working standard solutions after the reduction period was estimated as 3.0 M.

Method evaluation for selenium determination in fish

The analytical method performance for the determination of Se in fish by HG-AAS after acid digestion was evaluated based on the Appendix F of AOAC (2016) for analytical method validation. The limit of detection (LOD) and quantification (LOQ) were estimated by simultaneously analysing 11 separate blank samples. The following formula were used; LOD = $\bar{x}$ + 3SD and LOQ = 3LOD, in which $\bar{x}$ and SD were the average Se concentration calculated from 11 blank samples and their standard deviation values, respectively. The calibration curve was established based on the linear relationship ($y = ax + b$) between the Se concentrations (x) from 0.50 to 10.0 µg/L, and their corresponding atomic absorption values (y) measured at 196 nm. The repeatability and reproducibility were assessed through the calculation of relative standard deviations for six replicates (n = 6) within one day (RSD_r) and three separate days (RSD_R). The method accuracy was evaluated from the recovery tests of spiked samples and t-test of the certified reference material (CRM) of DORM-4 (National Research Council of Canada, NRC-CNRC) at the statistical significance level of 0.05 (Ellison *et al.*, 2009).

All investigation experiments and sample analyses were carried out in triplicates (n = 3) to assure the precision and accuracy of the results. The experimental data were calculated and analysed by Microsoft Office Excel 2016 (version 16.54, Microsoft 365 Subscription), then expressed as mean value ± standard deviation (SD). Relative standard deviations (RSDs) were calculated to evaluate and assure the measurement precision among runs.

Results and discussion

Effects of carrier solution concentrations

The analyte atomisation was carried out based on the principle of the flow injection hydride generation technique (Koreňovská, 2003), in which Se(IV) reacts with $NaBH_4$ reagent to produce the vapours of Se hydride species. Then, the formed hydride vapours would be transported to the atomisation unit (the heated quartz tube) by the Ar stream. The atomisation is the thermal decomposition (900°C) of the hydride vapours, which follows the mechanism of free radical reactions (1 to 4 below). Therefore, the atomisation efficiency depends not only on the temperature (intensities and stability), but also on the free radical densities. In the present work, the temperature of the quartz tube was set up and controlled by an electrical unit to ensure no remarkable temperature fluctuation during the analysis.

$$BH_4^- + 3H_2O + H^+ \rightarrow H_3BO_3 + 8H^* \quad (1)$$

$$4H_2SeO_3 + 3BH_4^- + 3H^+ \rightarrow 3H_3BO_3 + 3H_2O + 4SeH_2 \quad (2)$$

$SeH_2 + H^* \leftrightarrow SeH + H_2$ (3)

$SeH + H^* \leftrightarrow Se + H_2$ (4)

In this present work, HCl was used as the carrier solution to produce the medium for the hydride generation reaction. Figure 1 indicates that increasing HCl concentrations led to the increase in measured signals (the Abs increased from 0.107 to 0.153 when the HCl concentrations were from 1.5 to 4.5 M). This could have been due to the fact that the Se hydride species were generated with the help of Se(IV) derived from the reduction of Se(VI) to Se(IV) in HCl medium. However, from the HCl concentration of 3.0 M onwards, the measured signals did not exhibit any remarkable increase (0.1502, 0.1508, and 0.1516 for 3.0, 4.0, and 4.5 M, respectively). The lower measured signals and poorer precision observed for the low HCl concentrations from 1.5 to 2.5 M (%RSDs of 4.6 - 7.7%) might have been due the amount of HCl that was not enough to be favourable for the chemical reaction (4), thus leading to the fluctuation and instability of the measured signals. Low precision was obtained at 4.0 and 4.5 M HCl (%RSDs of 5.7 - 6.5%), and the highest precision was obtained at 3.0 M HCl (%RSD of 2.2%) as the carrier solution. The measured signal stability derived from high concentrations of HCl could be due to the following chemical reaction leading to the decreasing contents of Se(VI):

$3H^+ + HSeO_4^- + 2Cl^- \rightarrow H_2SeO_3 + H_2O + Cl_2$ (5)

Hence, it is vital to control the carrier solution concentration of 3 M HCl to assure the highest sensitivity and the most favourable repeatability for further investigations and experiments.

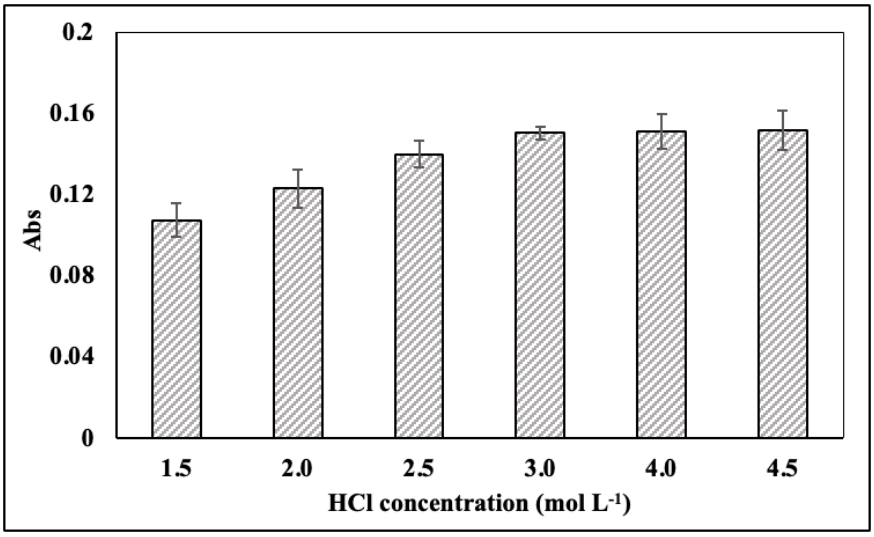

Figure 1. Effects of carrier solution concentrations on sensitivity.

Stability of Se(IV) after reducing steps in various HCl concentrations

For the determination of Se in fish by HG-AAS after acid digestion, HCl solution of 6.0 M could serve as a reducing agent without the addition of other reagents to effectively convert Se(VI) into Se(IV) (Koreňovská, 2003; Le *et al.*, 2013; Atasoy and Kula, 2022). Therefore, the solutions after the reducing period primarily contained HCl, and the stability of the reducing product of Se(IV) could depend on the contents of HCl in the media, *i.e.*, it was reported that the produced Se(IV) could be converted into Se(VI) during the sample preservation period. Therefore, the investigation into the stabilisation of Se(IV) in different HCl concentrations (1.0, 3.0, and 6.0 M) regarding the prolonged preservation time before the instrumental measurement should be carried out. In the present work, the stability of Se(IV) was assessed through the calculation of yields obtained from the hydride generation reactions between the Se(IV) solution after different preservation days and $NaBH_4$ in the medium of 3 M HCl as the carrier solution. The results in Figure 2 demonstrated that the decreasing hydride generation yields were observed for all of the three HCl concentrations, in which 6 M resulted in the strongest decrease, especially on day 4 onwards. This could have been due to the potential chemical reaction (5) as earlier mentioned, in which the equilibrium of this reaction would shift to the right side, thus resulting in the lower Se(IV), typically

when the concentrations of HCl are higher, *i.e*., 6.0 M HCl exhibited the lowest hydride generation yields. The situation was worse for smaller Se concentration (*i.e*., the yields obtained from 1.00 µg/L were lower as compared to those of 10.0 and 4.00 µg/L Se concentrations; Figure 2). Moreover, the presence of oxygen during the preservation might have contributed to the oxidation of Cl^- to Cl_2, thus resulting in the chemical equilibrium shifting to the side of decreasing Se(IV) contents. Figure 2 also indicates that there were no remarkable differences in the yields for HCl of 1.0 and 3.0 M. In order to match the concentration of the carrier solution (3.0 M HCl), the concentration of HCl in the Se working standard solutions and sample solutions should be kept at 3.0 M for further experiments and investigations. However, to reduce the risk of conversion between Se(IV) and Se(VI) after the reducing period, typically for long time preservation, the sample preparation should be conducted in batches, proceeding to the instrumental analysis right after or within the same day of the reduction step.

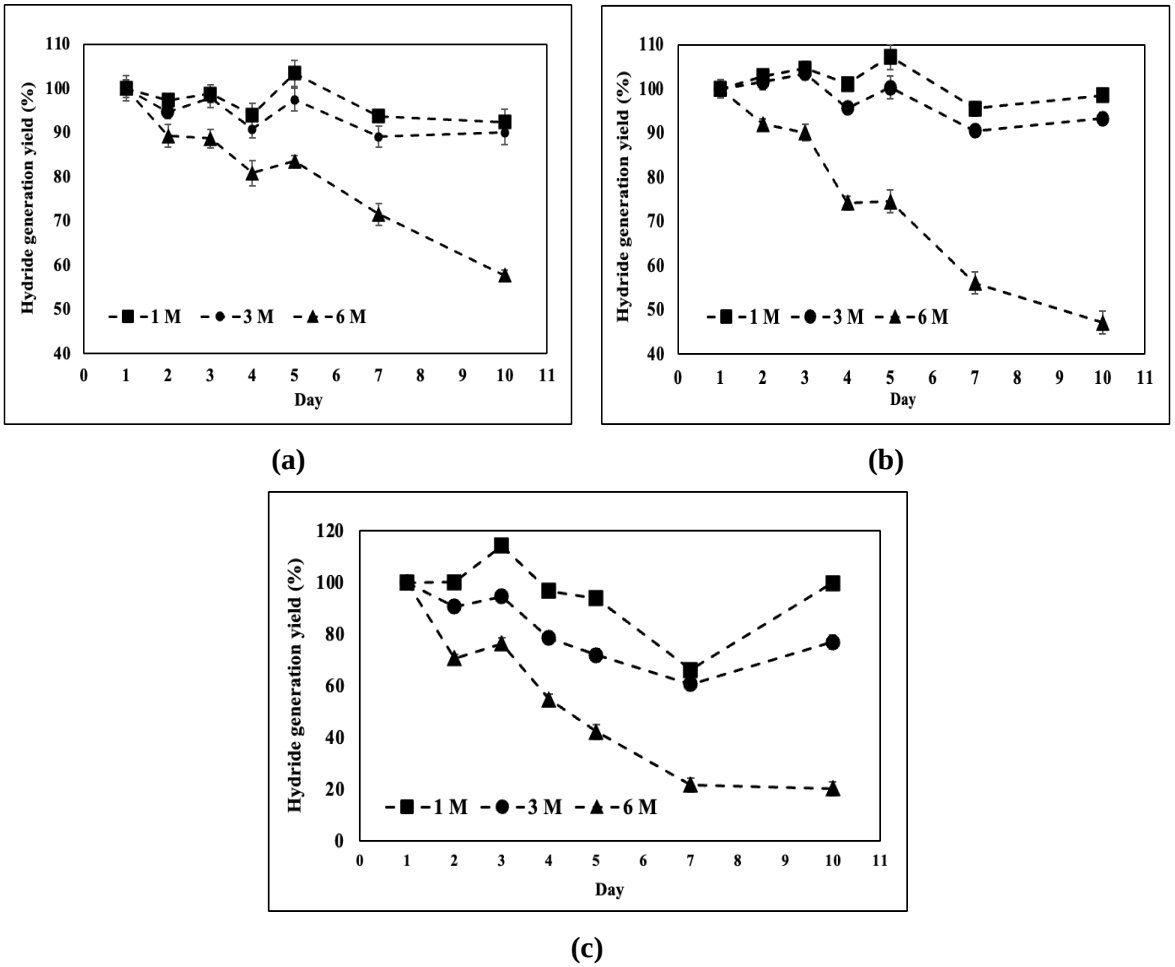

Figure 2. Stability of Se (IV) in different HCl concentrations: Se standards of **(a)** 10.0 µg/L, **(b)** 4.00 µg/L, and **(c)** 1.00 µg/L.

Analytical method performance for determining selenium in fish

The presence of chloride in the sample matrices and/or in the digestion reagents could result in the formation of easily volatile compounds of $SeCl_4$ (170 - 196°C) and $SeOCl_2$ (176°C) (Bye, 1983). However, in the present work, concentrated HNO_3 and $HClO_4$ as strong oxidising reagents were employed during the sample preparation procedure, which could effectively transform all Se species into their high oxidation state of Se(VI) to keep them present as non-volatile compounds, thus minimising the risks of analyte loss (Bohrer *et al.*, 2007). After the sample digestion, HNO_3 and $HClO_4$ were eliminated to reduce the effects of these oxidising residues on the reactions during the reducing period. Additionally, gentle heating was employed for the acid elimination step to minimise the risks of analyte loss due to certain volatile Se species (Hershey *et al.*, 1988; Le *et al.*, 2013).

The analytical method for the determination of Se in fish by HG-AAS after acid digestion by the concentrated HNO_3 and $HClO_4$ mixture was evaluated based on the guidance and requirements in the Appendix F of AOAC (2016). The limits of detection and quantification (LOD and LOQ) were estimated as 0.25 and 0.75 µg/L, respectively, which were compatible with the actual concentrations of Se in fish, *i.e*., varying in wide ranges and relatively high contents, up to hundreds and thousands of µg/kg or parts per billion (ppb) (Meche *et al.*, 2010; Azad *et al.*, 2019; Atasoy and Kula, 2022). Moreover, the estimated LOD was comparable with several other publications for Se analysis by AAS, *e.g*., 0.472 µg/L (Atasoy and Kula, 2022) and 0.650 µg/L (da Luz Potes *et al.*, 2019) without any preconcentration steps. The quantification of Se in fish was carried out based on the establishment of the calibration curve, $y = 0.0126x + 0.0005$. Good linearity was achieved due to the high correlation coefficient (R^2) of 0.9999 (*i.e*., $0.995 \leq R^2 \leq 1$ for linear regression) (Appendix F of AOAC (2016)). The precision was assessed through the intra-day ($\%RSD_r$) and inter-day analyses ($\%RSD_R$), and these obtained values (namely 1.9 and 3.5%, respectively) were favourable with the Appendix F of AOAC (2016) for analytical method validation. *t*-test was used to evaluate the accuracy, in which the calculated *t* value was lower than the theoretical *t* value (2.046 *vs.* 2.571, $n = 6$, $p = 95\%$). This revealed no statistically significant differences between the certified and measured Se mean concentrations from the CRM of DORM-4 (3560 ± 340 µg/kg) and the current analytical method (3274 ± 42 µg/kg) with the recoveries of around 92%. Therefore, the proposed analytical method could be used for routine analysis and further research activities as a simple, commonly equipped, and low-cost approach.

Variability of selenium contents in tropical fish species

The evaluated analytical method was applied for determining the concentrations of Se in various fish samples belonging to two types of frozen marine and freshwater fish. To ensure accuracy, recovery tests by Se-spiked fish samples were conducted for both marine and freshwater fish. The Se contents in fish samples were estimated, and a similar Se standard amount was spiked into the sample matrices prior to the sample preparation. The results indicated that the recoveries ranged from 96.7 to 99.1% (Table 2) and from 95.1 to 99.1% for marine and freshwater fish, respectively. These obtained values were in agreement with the requirements by the Appendix F of AOAC (2016) for analytical method validation. As presented in Table 2 and Figure 3, the Se contents for all fish samples in the present work were under the final chronic value (7.91 mg/kg) set by the EPA (2004), thus indicating the safety for human consumption. Generally, the Se contents in marine fish were 4.5 - 9.5 times higher than those of freshwater fish (*i.e*., 1131.2 ± 1.6 to 2109.5 ± 2.0 µg/kg *vs.* 119.7 ± 1.4 to 472.1 ± 2.0 µg/kg) (Li *et al.*, 2017; Yang *et al.*, 2021). This could be due to the differences in water sources, living depths, sizes, and ages between marine and freshwater fish, thus leading to the differences in food sources and bioaccumulation capacities (Meche *et al.*, 2010; Qin *et al.*, 2015; Azad *et al.*, 2019; Atasoy and Kula, 2022). The marine fish collected in the present work had bigger size and longer living time than the freshwater fish (several years *vs.* over one year as reported by the suppliers). The larger sizes of the marine fish help them to exist in the middle of the food chain and able to eat smaller marine life in their habitat, then accumulate certain contents of Se in their bodies, typically for prolonged living time. This suggestion was supported by the descending Se concentrations found as swordfish > tuna > butterfish, in which swordfish exhibit the biggest size, followed by tuna and butterfish (see Tables 1 and 2). As compared to other publications for Se in marine fish, there were certain variabilities among different sample sites and areas, which supported the Se content differences due to various living conditions, *e.g*., water and food sources, *etc*. However, relatively high Se concentration ranges up to thousand(s) µg/kg dried weight were found in the present work and other availably found references (Cabañero *et al.*, 2004; 2005; 2007; Fernández-Bautista *et al*., 2022).

For freshwater fish, the Se contents exhibited a descending order: snakehead fish (399.1 ± 1.0 - 472.1 ± 2.0 µg/kg) > anabas (281.2 ± 1.7 -301.2 ± 1.6 µg/kg) > catfish (217.8 ± 1.4 - 259.4 ± 1.8 µg/kg) > basa fish (158.2 ± 1.3 - 161.1 ± 1.2 µg/kg) > mullet fish (119.7 ± 1.4 - 130.1 ± 1.3 µg/kg). Although the snakehead and basa fish had similar size (Table 1), the snakehead fish yielded nearly three times higher in Se contents than the basa fish. The variation might be due to the younger age of basa fish (8 *vs.* over 12 months) and the different food sources, in which the snakehead fish in the present work lived in the natural

Table 2. Contents of Se in fish samples (µg/kg dried weight).

Se concentration in frozen marine fish (µg/kg dried weight)									
Tuna-1	**Tuna-2**	**Tuna-3**	**Butterfish-1**	**Butterfish-2**	**Butterfish-3**	**Swordfish-1**	**Swordfish-2**	**Swordfish-3**	**Reference**
1859.2 (1.9)	1739.5 (2.1)	1811.3 (1.2)	1235.1 (1.8)	1131.2 (1.6)	1313.2 (1.7)	2109.5 (2.0)	2007.3 (2.1)	1999.5 (1.9)	The present work
1570 (60)	-	-	-	-	-	1130 (40)	-	-	Cabañero *et al.* (2004)
2200 (100)	-	-	-	-	-	2200 (200)	-	-	Fernández-Bautista *et al*. (2022)
42 - 352*	-	-	-	-	-	304 - 854*	-	-	Olmedo *et al*. (2013)
734	-	-	-	-	-	-	-	-	Plessi *et al*. (2001)
2320 (30)	-	-	-	-	-	2090 (40)	-	-	Cabañero *et al.* (2005)
1070**	640**	-	-	-	-	-	-	-	Annibaldi *et al.* (2019)
2000 (30)	-	-	-	-	-	1630 (40)	-	-	Cabañero *et al.* (2007)
Recovery (%)									
97.8 ± 0.9	97.3 ± 0.8	98.0 ± 0.9	97.3 ± 1.0	99.1 ± 0.9	96.7 ± 0.8	97.2 ± 1.0	98.2 ± 0.9	98.2 ± 1.0	This study

*wet weight; **different sampling sites.

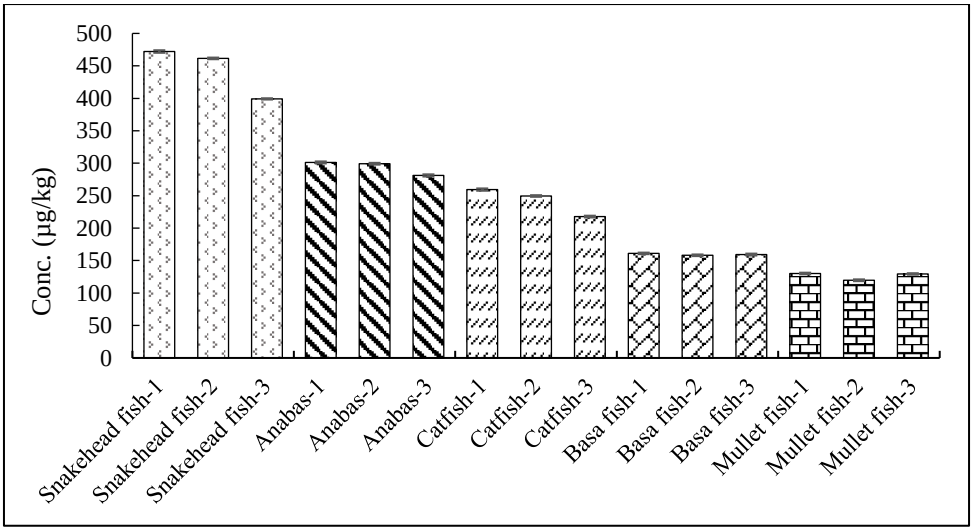

Figure 3. Concentrations of Se (µg/kg) in freshwater fish.

habitat, and the basa fish was fed by humans (farmed fish). The snakehead fish mainly eat insects, small shrimps, and other tiny fish while the basa fish only had one food source from humans, *i.e*., commercial fish feed. Moreover, the water sources for farmed fish such as basa fish could be more strictly regulated to minimise or eliminate the overdoses of potentially toxic elements and other contaminants. These results further supported the effects of size, age, habitat, and feeding activity on the concentrations of Se in fish bodies. The anabas in the present work exhibited the second highest Se content among the freshwater fish. This might have been due to the fact that anabas also lived in the natural environment, especially in the paddy fields. Therefore, the water and food sources could be affected by the application of fertilisers and other chemicals, *e.g*., pesticides, herbicides (Chen *et al*., 2002; Li *et al*., 2010). The catfish ranked third, which might have been due to their food sources and living conditions. Catfish live in the lower mud layer of rivers, which was reported to be the place for settling of different elements (Salim and Bloh, 1989; Chen *et al*., 2018). Besides, catfish are among omnivores which means they can eat insects, small snails, shrimps, and tiny fish, thus contributing to the Se bioaccumulation.

The study of Singhato *et al*. (2022) demonstrated the comparable Se concentrations for snakehead fish (438 ± 65 *vs.* 472.1 ± 2.0 µg/kg) and catfish (263 ± 9.3 *vs.* 259.4 ± 1.8 µg/kg) in Thailand (*vs.* the present work), which might have been due to the similar fish species and potentially living habitats. However, the obtained total Se concentration ranges for freshwater fish in Vietnam were lower than those in Argentina as reported by Kristan *et al.* (2013), with 119.7 - 472.1 *vs.* 666 - 1610 µg/kg. Moreover, another publication by Yang *et al*. (2021) determined Se in freshwater fish in Shandong province (China), and reported wider Se concentrations (61 µg/kg as the lowest and up to 891 µg/kg, wet weight). These results supported that the differences in Se concentrations in fish could result from the variations in species, size, age, and living condition (water and feed sources).

Conclusion

The analytical method in the present work employed acid digestion for sample preparation and HG-AAS for measurement of Se in fish samples. The effects of HCl concentrations as the carrier solution for the HG-AAS were investigated, which showed the highest sensitivity at 3 M. Moreover, sample preparation in batches and HG-AAS measurement within the same day after the reduction step should be carried out to ensure the maximum hydride generation yield. The method based on HG-AAS was evaluated regarding the Appendix F of AOAC (2016), and demonstrated good agreement for linearity (R^2 = 0.9999), precision (intra-/inter-day), and accuracy by *t*-test (p = 95%) and spiked samples. The Se contents were determined in 24 fish samples, in which Se concentrations of frozen marine fish were higher than those of freshwater fish. These results would contribute to the Se data for fish in Vietnam, and the method could be applied for the determination and control of Se in fish and other fish-related matrices.

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