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Quantification and Speciation of Mercury and Selenium in Fish Samples of High Consumption in Spain and Portugal

ANA I. CABAÑERO,[1] CRISTINA CARVALHO,[2] YOLANDA MADRID,[1] CAMILA BATORÉU,[2] AND CARMEN CÁMARA[1,*]

[1]Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain (E-mail: ccamara@quim.ucm.es); [2] Centro de Ciencias Farmaceuticas. Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto 1649-003 Lisboa, Portugal.

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ABSTRACT

Mercury (Hg) and selenium (Se) determinations were carried out to evaluate human exposure to those elements through fish consumption in Spain and Portugal. Atomic fluorescence spectroscopy (AFS) was applied in a cold vapor mode for total mercury quantification and was also hyphenated to gas chromatography (GC) to achieve the speciation of organomercurial species in fish samples. The results obtained show the highest concentration of Hg in swordfish and tuna (0.47 ± 0.02 and 0.31 ± 0.01 μg g^{-1}, respectively), and a much lower concentration in sardine, mackerel shad, and octopus (0.048 ± 0.002, 0.033 ± 0.001, and 0.024 ± 0.001 μg g^{-1}, respectively). The determination of alkyl mercury compounds revealed that 93–98% of mercury in the fish the three fish species with higher mercury content.

Total selenium concentration was high in sardine, swordfish, and tuna (0.43 ± 0.02, 0.47 ± 0.02, and 0.92 ± 0.01 μg g^{-1}, respectively), but low in mackerel shad and octopus (0.26 ± 0.01 and 0.13 ± 0.01 μg g^{-1}, respectively). Speciation of selenium compounds was done by high-performance liquid

* Author to whom all correspondence and reprint requests should be addressed.

chromatography in conjunction with inductively coupled plasma mass spectrometry (LC-ICP-MS). Selenomethionine (SeMet) was the only selenium compound identified in the fish samples with higher selenium content.

Among the fish species, studied sardine had the most favourable Se:Hg and SeMet:MeHg molar ratios; therefore, its consumption seems to be preferable.

Index Entries: Mercury, selenium, selenomethionine, speciation, fish.

INTRODUCTION

Mercury pollution has become a global problem because of its occurrence from natural and anthropogenic sources *(1),* and its detrimental effect on humans, making its detection of special concern among environmental pollutants.

Mercury toxicity is well known to be highly dependent on its chemical form *(2).* Methylmercury is the most important Hg species in terms of bioaccumulation and risk owing to its long biological half-life and accumulation through the food chain.

The major source of MeHg for humans is fish, because predatory species may preconcentrate 10,000–100,000 times the mercury concentration in water *(3).* Factors accounting for this magnification can be fish size and/or fat content *(4,5),* the protein affinity mechanisms *(6),* and the dissolved oxygen content of fish habitat *(7).*

The exposure to MeHg presents a risk for human health, owing to its teratogenic, immunotoxic, and, especially, neurotoxic effects *(3).* The lipophilic character of MeHg facilitates its absorption and the passage through hematoencephalic, placentary, and mammary barriers.

Selenium is an essential micronutrient for humans being a constituent of enzymes (such as glutathione peroxidase and 1-iodothyronine 5′-deiodinase). It is also well known for its potential in disease prevention *(8).* Among the effects reported in the area of health promotion, its role in decreasing cancer risk *(8,9)* and several types of diseases, such as cardiovascular, Keshan and Kashin–Bek diseases, as well as liver necrosis *(10,11),* should be stressed.

Selenium is also a well-known antagonist of mercury toxicity *(10,12).* The way in which selenium interferes with MeHg is still unknown, and several mechanisms have been proposed to explain this interaction; however, none of them is conclusive *(13).* Among the hypotheses, some are more likely to occur: (1) Se may promote a redistribution of MeHg from more sensitive organs (CNS, kidney) to others less sensitive (muscle); (2) competition of Se for the same receptors; (3) formation of complexes (such as tiemannite) *(14);* and (4) promotion of MeHg conversion into less toxic forms and prevention of oxidative damage *(15).*

Among the species of Se, selenomethionine is one of the species of Se of most interest for human health because it has the highest rate of absorp-

tion and retention in tissues, and the highest level of incorporation into enzymes and proteins *(10,16).* Furthermore, it represents the major nutritional source of selenium for higher animals and humans *(16).*

Mercury and selenium in foods have mostly been determined at the trace level using spectrophotometric techniques (ETAAS, HGAAS, CVAAS, HGAFS, and CVAFS), which are preferred because of their low detection limits *(17–19).* It is well known that the toxicity, bioavailability, and environmental mobility of metals are strongly dependent on on their chemical forms. Thus, analytical speciation has been gaining increasing importance, especially when organic and inorganic forms of the same element can be present in foods, because different forms of an element can have different toxic or protective effects in living organisms. To the best of our knowledge, never before has speciation of selenium and mercury been carried out on the same sample. Overall, food safety and nutritional quality depend on the determination of total levels as well of speciation of the trace elements that exist in foodstuffs.

Risk assessment and prevention of human exposure to MeHg is a strong objective in food safety but unfounded warnings to consumers should be avoided, because of the high nutritional value of fish. Instead, consumers should be provided a clear information, supported by research, about the risk of food consumption.

The main goal of this work was to measure the concentration of mercury and selenium, including MeHg and SeMet, in fish commonly consumed in Spain and Portugal.

EXPERIMENTAL PROCEDURE

Instrumentation

An atomic fluorescence spectrometer (AFS, Merlin 10.023, P.S. Analytical Ltd., Orpington, Kent, UK) was used to determine the total mercury content. Mercury vapor was generated in a flow injection system consisting of a multichannel peristaltic pump, a six-way injection valve, and a gas–liquid separator.

A gas chromatograph (Perkin Elmer, Ltd. model 8410, England) was hyphenated to the AFS detector for the speciation of organomercury compounds. Separation of organomercury compounds was carried out in a gas chromatograph with an on-column injector. The chromatograph was fitted with a non-polar capillary fused silica column SGL-1 (15 m × 0.53 mm id) coated with 1.5 μm dimethylpolysiloxane (Sugelabor S.A. Spain). A pyrolyzer unit 10.558 (P.S., Analytical, Kent, UK) was used as the interface between GC and AFS in order to convert the organomercurial compounds to atomic mercury vapor. An atomic fluorescence spectrometer (AFS, Excalibur, P.S. Analytical Ltd., Orpington, Kent, UK) was used to determine the total selenium content. Selenium hydride was

generated in a flow-injection system consisting of a multichannel peristaltic pump, a six-way injection valve, and a gas–liquid separator. An inductively coupled plasma mass spectrometer (ICP-MS, HP-4500 Plus, Tokyo, Japan) fitted with a Babington nebulizer and a Scott double-pass spray chamber was used for selenium detection after chromatographic separation. A CM4000 HPLC pump (Milton Roy, Riviera Beach, FL, USA) fitted with a six-port sample injection valve (model 7725i, Rheodyne) with a 100 µL injection loop was used for chromatographic experiments. Separations were carried out in a Hamilton PRP-X200 (10 mm, 250 × 4.1 mm id) (Reno, NV, USA) for cationic chromatography. For molecular-weight fractionation, 10 kDa cut-off filters (Millipore, Bedford, MA, USA) and an Eppendorf (Hamburg, Germany) Centrifuge 5804, F34-6-38 were used.

Reagents

All reagents were of analytical grade and were used without further purification. Mercury standard solutions were prepared by dilution of a stock mercury (II) solution (1000 mg L^{-1}) (Merck) in deionized Milli-Q water (Millipore, Ohio, USA). Standard stock solutions of 1000 mg L^{-1} of methylmercury chloride, dimethylmercury, and ethylmercury chloride (Alfa Aesar, Karlsruhe, Germany) were prepared in methanol (HPLC grade, Scharlau). These solutions were stored in amber vials at –18°C and diluted with methylene chloride (HPLC grade, Scharlau) to obtain working standards. Standards were prepared daily to reduce the risk of mercury volatilization.

Inorganic selenium solutions were obtained by dissolving sodium selenite and sodium selenate (Merck, Darmstadt, Germany) in deionized Milli-Q water (Millipore). Selenocystine and selenomethionine (Sigma Chemicals, St. Louis, MO, USA) were dissolved in 3% hydrochloric acid and deionized Milli-Q water, respectively. Trimethylselenonium chloride was synthesized in our laboratory following the procedure of Palmer et al. *(20)*. Stock solutions of 10 mg L^{-1} were stored in the dark at 4°C. Working standard solutions were prepared daily by dilution.

Stannous chloride 3% (w/v), used as a reducing agent, was prepared by dissolving stannous chloride dehydrate (Merck, Darmstadt, Germany) in concentrated hydrochloric acid and diluted to volume with ultra-pure water to a 3 mol L^{-1} concentration. Reagent solution for the reduction was prepared daily to reduce hydrolysis of stannous chloride and maintain the efficiency of mercury reduction.

An acidified solution of potassium bromide [18% (m/v) in 0.5% (v/v) H_2SO_4 (Scharlau)], 1 mol L^{-1} copper (II) sulfate, and 0.01 mol L^{-1} Na_2SO_4, were used in sample preparation for mercury speciation.

For HG-AFS studies, 1% sodium borohydride solution in 0.3% sodium hydroxide (Merck) was prepared. For LC-ICP-MS studies, the mobile phase was 4 mmol L^{-1} pyridine formate in 3% methanol. The elu-

ent was prepared by diluting commercial pyridine (Merck) with distilled water and adjusting the pH to 2.8 or 4.7 with formic acid (Merck). HPLC-grade methanol was purchased from SDS (Barcelona, Spain). For the enzymatic hydrolysis procedure, TRIS-HCl buffer (pH 7.5) and the non-specific protease XIV *(Streptomyces griseus)* (Merck) were used.

H_2O_2 (35%) from Panreac and HNO_3 (65%) were used for acid digestion of samples. Helium c-50 was used as a carrier gas and argon c-50 was used as a make up gas and sheath gas at the transfer line and the AFS, respectively (Carburos Metálicos, Spain).

SAMPLES

Fish samples: mackerel shad *(Trachurus trachurus)*, octopus *(Octopus vulgaris)*, swordfish *(Aphanopus carbo)*, sardine *(Sardina pilchardus)*, and tuna (*Thunnus* spp.) were collected on docks (Sesimbra, Portugal) just before delivery to consumers or purchased in a local market following the recommendations of European Commission 2001/22/CE (JO CE, 2001). The skin and bones were removed, then the edible (muscle) portions of fishes were immediately blended and frozen at –70°C. Analyses were performed avoiding UV radiation, owing to its detrimental effects on organomercurials. The results were validated using two certified reference materials: tuna tissue CRM-463 certified for methylmercury (2.85 ± 0.16 μg g^{-1}), from the Community Bureau of Reference of European Commission (BCR), and Antarctic krill Murst-ISS A22, certified for selenium (7.37 ± 0.91 μg g^{-1}), from Institute for Reference Materials and Measurements.

PROCEDURES

Figure 1 briefly describes an overview of the procedure for total mercury and selenium determination and further speciation.

Total Mercury Quantification

Fish tissues (0.5 g of wet fish or 0.2 g of dry fish) were digested with 2–5 mL of nitric acid and 0.5 mL of hydrogen peroxide in an analytical microwave oven at 43% power output. The pressure was kept at 20 psi during 15 min, increased to 40 psi during 30 min and kept during 1 h at 85 psi. Total mercury concentration was determined by both external and standard addition calibrations of the signal obtained by the continuous mercury cold vapor system connected to AFS equipment. A flow rate of 2.5 mL min^{-1} (3 *M* HCl) and a similar flow rate of the reductant solution (3% $SnCl^2$ in 15% HCl) were used to generate the mercury cold vapor.

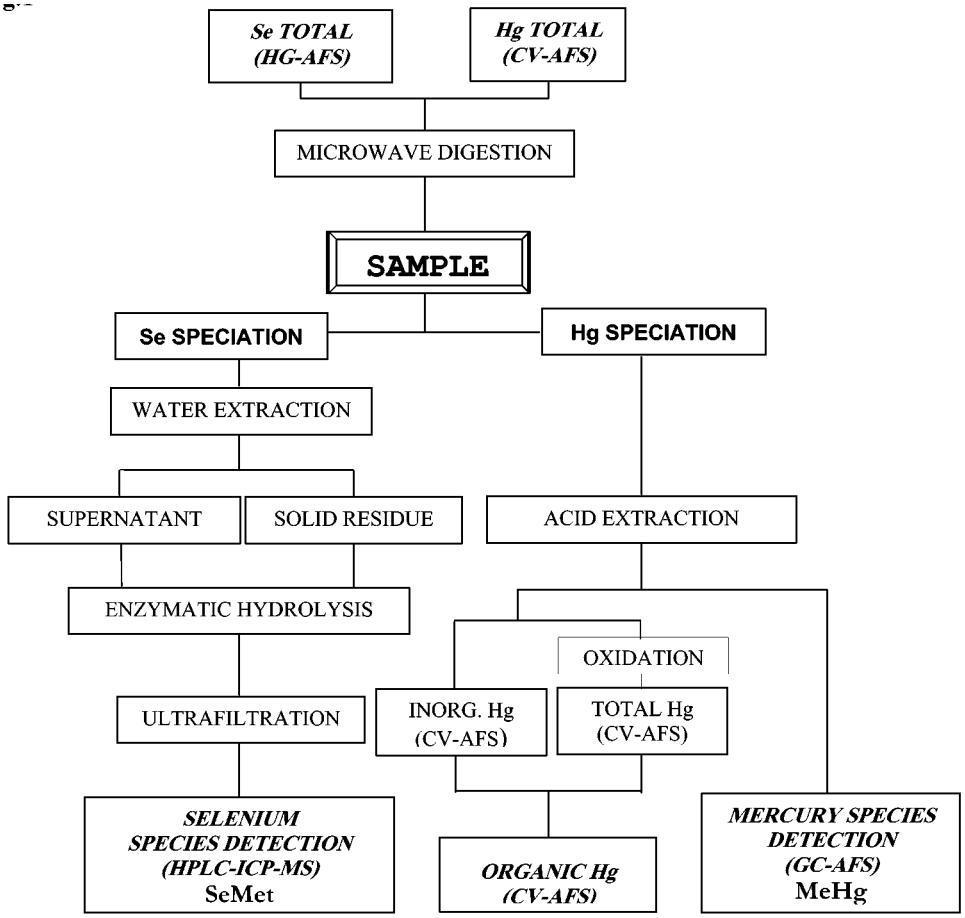

Fig. 1. Flow chart of the sample treatments used for mercury and selenium speciation in fish samples.

Mercury Speciation

Acid Extraction

Five milliliters of 5 mol L^{-1} hydrochloric acid were added to a portion (300 mg) of fish tissue and sonicated for 5 min.

Mercury Speciation

After neutralization, mercury species of the hydrochloric acid extracts were converted into their bromide derivates (RHgBr) by the addition of 3 mL of a solution containing 18% (w/v) potassium bromide, 5%(w/v) sulfuric acid, and 1 mL of 1 mol L^{-1} copper sulphate.

Extraction of organomercury species into the organic phase was carried out by adding 5 mL of methylene chloride and shaking the solution for 5 h. A 4 mL aliquot of the organic solvent layer containing the extracted

organomercury was transferred to a glass vial, and a 1 mL of 0.01 mol L^{-1} sodium thiosulfate solution was added. The solution was mixed for 20 min and subsequently centrifuged at 1575*g*.

An aqueous layer (800 µL) was placed in a 3 mL polyethylene vial and 300 µL of $KBr/CuSO_4$ and 300 mL of CH_2Cl_2 were added. Each vial was manually shaken for 1 min, centrifuged, and 0.1–0.2 mL of the organic solvent was extracted.

Detection

Speciation of organic mercury was attained on the organic extract with the coupling GC-pyrolyser-AFS.

Helium with a flow rate of 10 mL min^{-1} was used as the carrier gas. The temperatures were 250°C for the injector and 40°C with a ramp of 15°C min^{-1} until 200°C for the oven.

For the AFS detection, argon was used as make-up gas and sheath gas at flows of 60 and 300 mL min^{-1}, respectively.

Total Organomercury Determination

After acid extraction total organomercury content in the supernatants was determined by difference between its total mercury content (after digestion with HNO_3 and H_2O_2) and its inorganic mercury content, by using stannous chloride as a selective reductant.

Total Selenium Quantification

The samples for selenium determination followed the same acid digestion as mentioned for total mercury quantification. Se (VI) was reduced to Se (IV) by adding concentrated hydrochloric acid (6 mol L^{-1} final concentration) to the digest and heating at 95°C for 1 h. The solutions were then diluted to 25 mL with Milli-Q-water.

Total selenium concentration was determined by the continuous selenium hydride system connected to AFS equipment. A flow rate of 1.5 mL min^{-1} (3 *M* hydrochloric acid) and a similar flow rate of the reductant solution (1% sodium tetrahydroborate w/v) were used to generate the selenium hydride.

Selenium Speciation

Portions (200 mg) of dried fish were enzymatically hydrolysed following a previously developed method *(21)* (hydrolysis of water-soluble fraction and its solid residue at pH=7.5), but using the non-specific protease (*Streptomyces griseus,* Pronase E) instead of Subtilisin.

In order to enhance the clean up, the extracts were processed through 10 kDa mass cut-off filters. Finally, the filtrates were diluted to 10 mL and analyzed by cation exchange chromatography coupled to ICP-MS, under the following operating conditions: coolant Ar flow rate: 15.0 L min^{-1};

auxiliary Ar flow rate: 1.1 L min^{-1}; nebulization Ar flow rate: 1.3 L min^{-1}; sample flow rate: 1.0 mL min^{-1}; nebulizer type: Babington. For this purpose a Hamilton PRP-X200 cationic exchange column using 4 m*M* pyridine at pH 2.8 and 4.7 as a mobile phase was evaluated. The analytical peaks were evaluated in terms of peak area by the standard addition method at *m/z* 82 and 78.

RESULTS AND DISCUSSION

Total Mercury Determination

Total mercury content of the samples was determined by FI-CV-AFS in order to evaluate the mercury exposure through fish consumption. Mercury concentration in marine tissue showed the following gradation: octopus (0.024 μg g^{-1}) < mackerel shad (0.33 μg g^{-1}) < sardine (0.046 μg g^{-1}) < tuna (0.31 μg g^{-1}) < swordfish (0.47 μg g^{-1}) (Table 1). Such variability might be explained by the interference of biotic parameters such as age, size, sex, metabolism, and feeding habits *(4–6)* that affect the bioaccumulative process of Hg in fish.

All these values are within the European Commission Regulations 466/2001 and 221/2002 *(22,23),* which forms part of EC food hygiene legislation, and sets the maximum limit for mercury in whole fresh fish at 0.5 mg kg^{-1}, except mainly for predatory species, which may have higher mercury concentration (1.0 mg kg^{-1}). The value for mercury for these three species was comparable with other Hg data found in fish *(24–26).*

The accuracy of this method was evaluated by analyzing a marine tissue reference material (CRM 463, tuna fish). Because, at 95% confidence level, no significant differences were detected between the certified value and the experimental one, the method used was considered accurate for total mercury determination.

Mercury Species Analysis

Several procedures based on alkaline digestion have been used for the analysis of mercury speciation, but occasionally have shown overestimation of methylmercury concentration *(27–28).* Therefore, to perform mercury species extraction, acid extraction (hydrochloric acid) combined with an ultrasound bath extraction was selected. Several variables, such as acid concentration (0–7 *M*), volume of extractant (0–7 mL), reagents, and sonication exposure time (0–15 min), were previously optimized.

Once total mercury extraction was carried out, determination of inorganic and total mercury was achieved in the acid extract by CV-AFS. The results obtained showed that organomercury compounds comprised more than 94% from the total mercury occurring in fish samples. This may be explained because of the concurrent MeHg bioamplification phenomena

Table 1
Total Mercury Concentrations (μg g^{-1}) Found in Fish Samples by CV-AFS

Sample	Moisture (%)	Total Hg fresh weight (μg g^{-1})	Total Hg dry weight (μg g^{-1})	Total Se fresh weight (μg g^{-1})	Total Se dry weight (μg g^{-1})	Se:Hg Molar ratio
Mackerel shad	80	0.033 ± 0.001	0.172 ± 0.007	0.26 ± 0.01		
Octopus	79	0.024 ± 0.001	0.111 ± 0.005	0.13 ± 0.01		
Swordfish	78	0.47 ± 0.02	2.05 ± 0.04	0.47 ± 0.02		
Sardine	76	0.048 ± 0.002	0.21 ± 0.01	0.43 ± 0.02		
Tuna	60	0.31 ± 0.01	0.73 ± 0.03	0.92 ± 0.01		
CRM-463[a]	3	--	2.87 ± 0.07	--	--	
Murst-ISS A2[b]	2	--	--	--	7.42 ± 0.5	

[a] Certified value: 2.85 ± 0.16 μg g^{-1}.
[b] Certified value: 7.37 ± 0.91 μg g^{-1}.
[c] Results expressed as mean value ± standard deviation, n=6.

through the tropic chain and the high specificity of the intestine wall of fishes toward MeHg absorption *(29).*

In order to evaluate the organomercury compounds, after applying the HCl extraction procedure, mercury speciation of the extracts with gas chromatography coupled to pyrolysis with atomic fluorescence detection was carried out.

A chromatographic analysis was performed on different fish samples and only MeHg was detected. This fact is in good agreement with the literature, where MeHg is the only organomercury compound found in fish *(1).*

The results of the speciation analysis for swordfish, tuna, and CRM-463 were 1.8 ± 0.1 μg g^{-1}, 0.72 ± 0.05 μg g^{-1}, and 2.8 ± 0.02 μg g^{-1}. It can be stated that MeHg was found to be the dominant Hg species in all the samples analyzed. Depending on the fish, the amount of mercury found varies, but the percentage of MeHg is higher than 93% in all the samples. This fact was in agreement with the value provided previously for the total organomercury compound.

The proposed HCl extraction GC-AFS method provided a limit of detection for methylmercury of 1.2 pg, calculated as three times the standard deviation of 10 blank measures.

The method was validated by the analysis of the standard reference material, CRM 463 tuna fish. No significant differences were found between the certified value and the one provided by the acid leaching method (GC-AFS) at 95% confidence level.

Total Selenium Determination

Total selenium content of the fish samples was determined in order to evaluate the Se exposure of fish consumers. The results obtained are shown in Table 1. Selenium concentration in the muscle tissue did not vary to the same extent as the mercury levels did. Mean selenium concentration in marine tissue ranged from 0.13 μg g^{-1} in octopus to 0.92 μg g^{-1} in tuna. Swordfish and sardine had a similar Se content; however, tuna doubled this amount. Selenium concentration for these three species was comparable with other Se data found in fish *(26,30,31).*

Even though swordfish and sardine had similar Se content, the molar ratio Se:Hg varies from 3 (swordfish) to 22 (sardine).

The accuracy of the method was evaluated by analyzing a marine tissue reference material (Murst-ISS A2). Because, at 95% confidence level, no significant differences were detected between the certified value and the experimental one, the method used was considered accurate for total selenium determination.

Selenium Species Analysis

To date, very few Se speciation studies have been carried out in marine organisms, most of them concerning with oysters *(21)*, cod *(30)*,

and tuna *(31)*. However, no data about $Se_{species}$:$Hg_{species}$ ratio in fish has been reported to our knowledge.

Generally, the use of enzymatic hydrolysis processes has shown better results in the release of selenium species from biological solid samples *(21)* than basic (tetramethylammonium hydroxide) or acid (hydrochloric acid) hydrolysis that led to selenium species degradation *(32,33)*. Because of this, selenium species extraction was performed following an enzymatic procedure.

Se may be present in food sample as inorganic forms (selenite or selenate) or as seleno amino acids (free or forming part of the protein structures). The aqueous extraction may free the weakly bound inorganic Se and soluble seleno amino acids. On the other hand, the enzymatic hydrolysis of biological samples produces free amino acids and peptides of different sizes because of the cleavage of peptide bonds in proteins.

After an aqueous extraction, it was found that the percentage of soluble selenium was between 6% and 8% of total concentration, the rest of the selenium was found in the solid fraction.

After the enzymatic hydrolysis, total selenium contents were analyzed before and after passing through the 10 kDa cut-off filters in order to evaluate if there were selenium compounds retained in the filters. For swordfish, selenium recoveries ranged from 90% to 97%, which indicates that the molecular weight of most of the selenium species extracted during the hydrolysis are lower than 10 kDa. When tuna and sardine were analyzed, the recovery ranged from 65% to 75%, respectively, which means that the hydrolysis was not as effective in breaking down the peptides or proteins in smaller fractions as it was for swordfish, so some selenium may remain in peptide form. This speculation stems from the knowledge that in the enzymatic hydrolysis of proteins, some peptide bonds can remain intact depending on the cleavage specificity of the enzyme *(34)*. Therefore, in this type of fish, a selenium fraction with a molecular weight higher than 10 kDa remained after the enzymatic hydrolysis step.

To ensure that no selenium compounds lower than 10 kDa were retained in the filters, we determined the total selenium content in the extracts (spiked with the standards) after filtration. The results, with an average recovery of 95–98% of total selenium, showed that selenium losses did not occur with this sample treatment.

Qualitative Analysis of Selenium Species

Some analytical methods for separation and quantification for organic and inorganic selenium species have been proposed *(35)*. Generally, ionic exchange liquid chromatography is used for the separation of seleno amino acids and inorganic selenium species, owing to a number of potential benefits [minimal preparation of liquid samples, separation at ambient temperatures avoiding the risk of thermal decomposition of labile compounds *(36)*, and remaining of their ionic properties in a wide pH range].

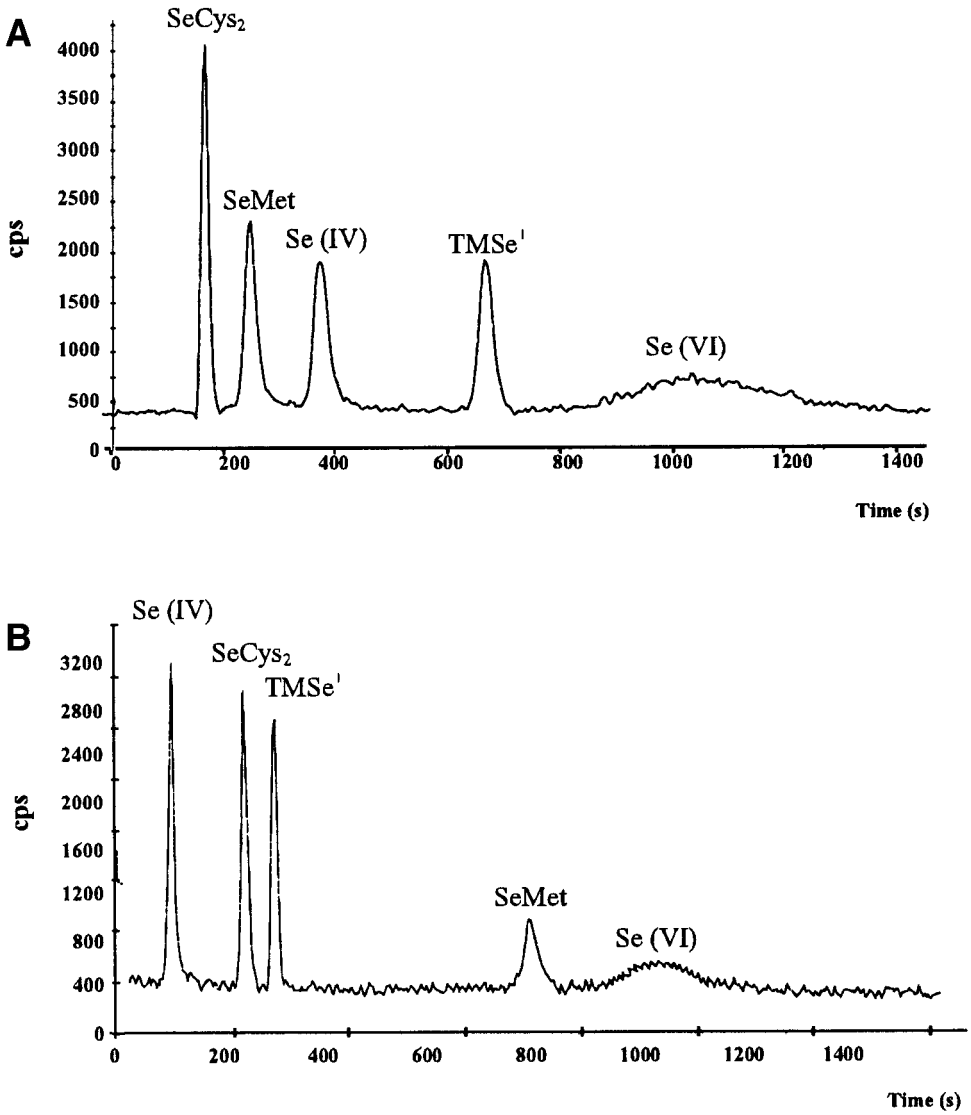

Fig. 2. Chromatograms of 10 µg L^{-1} of Se species obtained for cationic exchange chromatography at two pH values: **(A)** 4.7 and **(B)** 2.8.

In this study, five standard Se compound and two mobile phases were tested for Se separation by a cationic exchange column. If a species is identified under two different chromatographic conditions, its identity can be more certainly stated. Because of this, different experiments were carried out to provide good resolution for standard solutions using two different mobile phases at different pH values. A pH value studied previously (2.8) *(19)* and a new one (4.7) were used. A standard chromatogram for both pHs is shown in Fig. 2 and the limits of detection (LODs) of the

Table 2
Limits of Detection of the Cation Exchange Chromatographic Methods used for Selenium Speciation Analysis
(Injection Volume 100 µL)

Species	pH 2.8		pH 4.7	
	LOD (Se/ng)	RSD (%)	LOD (Se/ng)	RSD (%)
$TMSe^+$	0.1	3	0.1	2
$SeCys_2$	0.1	3	0.2	2
SeMet	0.4	3	0.3	3
Se(IV)	0.2	2	0.15	2
Se(VI)	0.5	4	0.6	3

two chromatographic methods are shown in Table 2. Under the optimal chromatographic conditions, it is possible to identify these species within 20 minutes.

A chromatographic analysis was performed on three different fish samples. Figures 3 and 4 show the chromatograms obtained for these samples (water-soluble fraction and solid residue) analyzed. Two peaks can be differentiated in each of the evaluated samples. The first peak was unidentified; it could not be attributed to any of the selenium species tested, so it could correspond to any anionic selenium species that elutes in the dead volume. The second peak was identified as SeMet, the only seleno amino acid found in both extracts and the dominant Se-species in the three samples. The identification of the peaks was carried out by the spiking procedure. The same chromatographic profiles were obtained by the two chromatographic methods used (at pH 2.8 and 4.7).

According to the literature, SeMet is the main form of Se found in food *(16,37,38).* Therefore, the majority of Se in plant and animal material consumed by humans will be in the form of SeMet *(39).* Because SeMet cannot be synthesized by higher animals and humans, it could have beneficial physiologic effects not shared by other selenium compounds and meet the criteria of an essential amino acid *(16).* Only SeMet is incorporated into body proteins, and this allows Se to be stored in the organism and reversibly released by normal metabolic processes, thus offering an advantage over other Se compounds *(37).* In addition, organic Se appears to be more bioavailable and maintains higher post-supplementation levels *(38).*

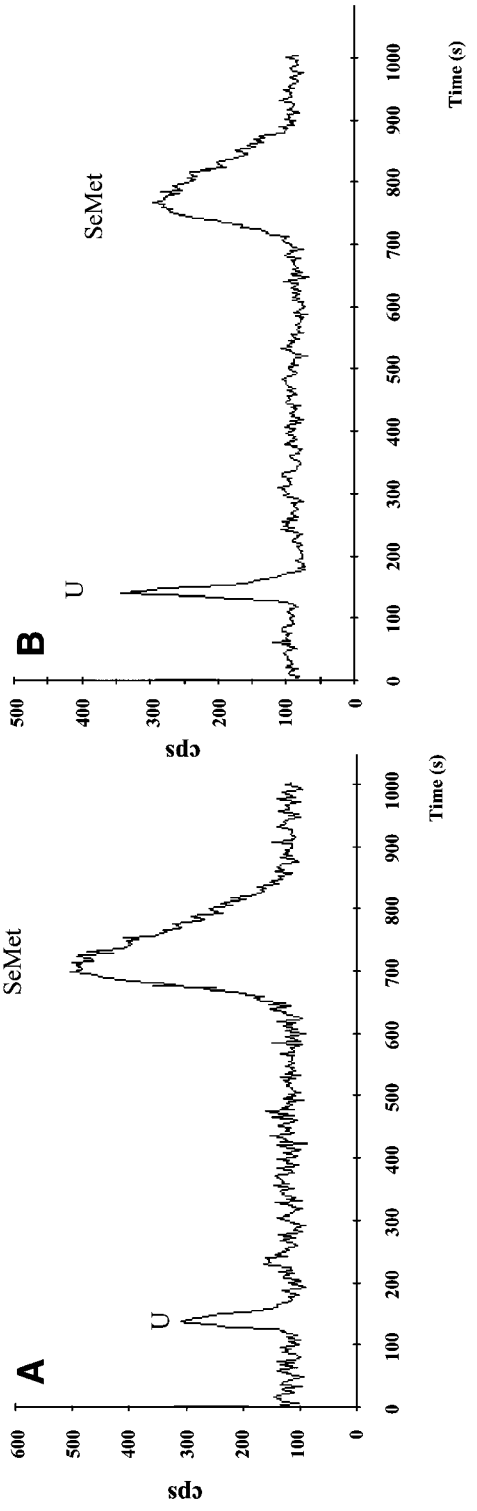

Fig. 3. Chromatograms of Se species found in sardine after enzymatic hydrolysis and 10 kDa ultrafiltration in: the solid residue **(A)** pH 2.8, **(C)** pH 4.7, and in the water-soluble fraction **(B)** pH 2.8 and **(D)** pH 4.7. U= unidentified Se species.

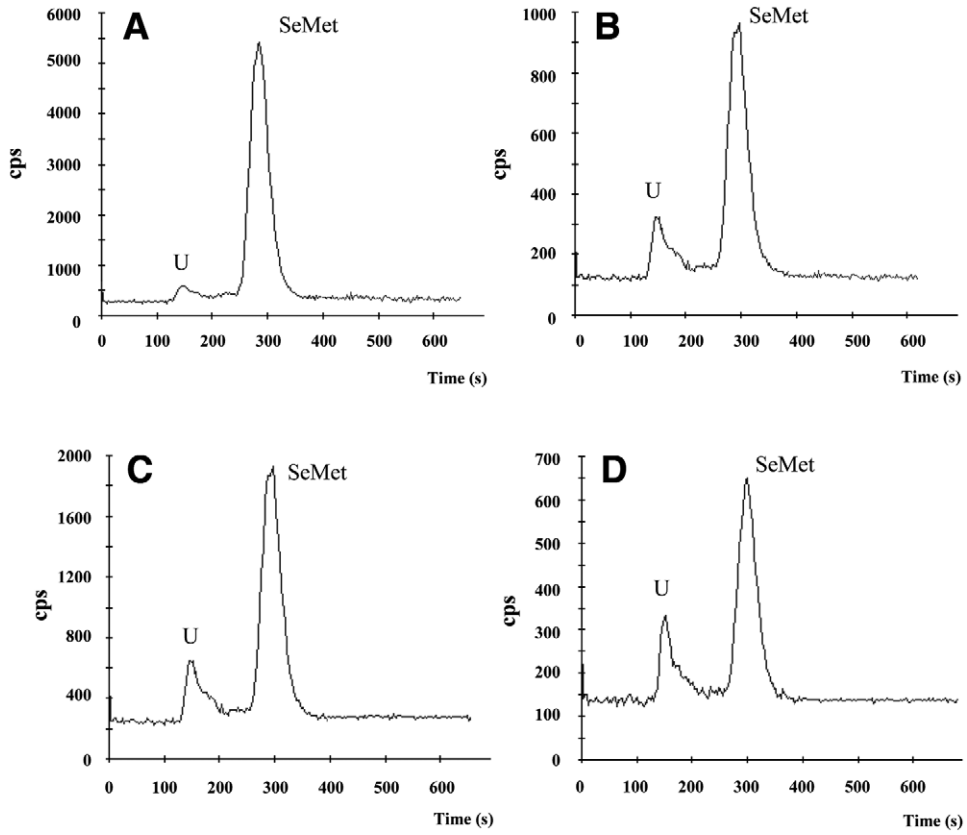

Fig. 4. Chromatograms of Se species found after enzymatic hydrolysis hydrolysis and 10 kDa ultrafiltration in the solid residue of: **(A)** swordfish, **(C)** tuna and in the water-soluble fraction of **(B)** swordfish and **(D)** tuna. U= unidentified Se species. Cationic exchange chromatography at pH 4.7.

Quantitative Analysis of Selenium Species

The results of speciation analysis of fish samples are shown in Table 3. The amount of SeMet varied depending on the type of fish. In the swordfish, 93% of the total Se has been found to be SeMet, on the other hand, tuna and sardine present lower percentage of SeMet (46% and 28%, respectively). The value for SeMet for these species was comparable with other SeMet data found in marine samples [e.g, oyster (47%)*(21)*] Consequently, the selenium levels found may not only help to achieve the recommended daily amount but provide a rich dietary source of SeMet.

In spite of the fact that the highest SeMet content has been found in the swordfish, it can also be seen that the highest value of the SeMet:MeHg ratio (6.3), as well as the Se:Hg ratio (22) is for sardine, and the lowest one for swordfish (3).

Table 3
SeMet Concentration Found Both Soluble and Non-soluble Fractions after Enzymatic Hydrolysis of Fish Samples

Sample	SeMet (water-soluble fraction) ($\mu g\ g^{-1}$)	SeMet (solid residue) ($\mu g\ g^{-1}$)	Total SeMet ($\mu g\ g^{-1}$)	SeMet:MeHg Molar ratio
Swordfish	0.090 ± 0.007	1.86 ± 0.12	1.95 ± 0.12	2.7
Sardine	0.11 ± 0.01	0.41 ± 0.02	0.51 ± 0.02	6.3
Tuna	0.11 ± 0.01	0.96 ± 0.03	1.07 ± 0.03	3.8

[a] Results expressed as mean value ± standard deviation, *n*=6.

CONCLUSIONS

The goal of this study was to obtain information about mercury contamination in fish highly consumed in Spain and Portugal to establish future actions on exposure assessment resulting from fish ingestion.

The levels of total mercury found in the fish samples analyzed vary to a large extent depending on the species, and are below the maximum level allowed by the European legislation. The results also showed that more than 93% of the total Hg occurring in fish samples was methylmercury.

On the other hand, it can therefore be stated that the average concentrations of selenium did not vary to the same extent as the mercury levels did, and that they are important to ensure the recommended daily amount.

SeMet was the only selenium compound found in the three fish species (sardine, swordfish, and tuna) after an enzymatic hydrolysis process. Nevertheless, the SeMet:MeHg ratio in the different species of fish differs substantially.

The ratio Se:Hg varied from 3 (swordfish) to 22 (sardine) with a more favorable ratio between SeMet and MeHg in the sardines. Sardine consumption thus seem to be preferable over tuna and swordfish.

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