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Fast determination of arsenic, selenium, nickel and vanadium in fish and shellfish by electrothermal atomic absorption spectrometry following ultrasound-assisted extraction

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Abstract

Ultrasound-assisted extraction of As, Se, Ni and V from fish and shellfish has been applied as a fast and reliable sample pre-treatment method for accurate determination of the four elements by electrothermal atomic absorption spectrometry with Zeeman (As, Se) or Deuterium (Ni, V) background correction. A multivariate optimization approach has been employed for establishing the effect of variables influencing the extraction process. Under suitable conditions, quantitative extractions occurred from a 10 mass sample (particle size <100 μm) suspended in 1.5 mL of acidic extractant (0.5 or 3% v/v HNO_3) and subjected to high intensity ultrasound (50% amplitude; 3 min). The method was successfully validated against the following certified reference materials: NRCC-DORM-2 dogfish muscle, NRCC-DOLT-2 dogfish liver, NRCC-TORT-2 lobster hepatopancreas, NIST-SRM 1566b oyster tissue and BCR 627 tuna fish. The following seafood samples were analyzed: hake (*Merluccius merluccius*), sole (*Solea solea*), clam (*Venerupis rhomboides*), prawn (*Panaeus kerathurus*), cuttlefish (*Sepia officinalis*), shrimp (*Palaemon elegans*), razor shell (*Ensis ensis*), cockle (*Cardium edule*), Mussel (*Mytilus galloprovincialis*), edible crab (*Cancer pagurus*), meagrin (*Lepidorhombus whiffiagonis*). The concentration ranges (μg/g, dry weight) for the elements determined were as follows: As (12.6–190), Se (0.73–2.34), Ni (2.94–46) and V (0.82–5.14). The detection limits (LODs), defined as $3s/m$ (s being the standard deviation of 10 blank and m the slope of the calibration graph), in dry tissue were 0.6, 0.3, 0.2 and 0.4 μg/g for As, Se, Ni and V, respectively. Between-batch precision was expressed as relative standard deviation from three separate extractions was in the range 3–10%.

Keywords: As; Se; Ni; V; Fish and shellfish; High intensity ultrasonic extraction; Atomic absorption spectrometry

1. Introduction

The harmful effects of heavy metals and metalloids to biota in the marine environment have been recognized. Fish and shellfish are good bio indicators of trace element contamination in the marine environment since they occupy different trophic levels and can display large bioaccumulation factors (Neff, 2002). The consumption of contaminated seafood is the responsible for an important route of human exposure to toxic elements, and consequently, several agencies and organizations throughout the world such as the US Food and Drug Administration (US FDA, 1993), Food and Agriculture Organization (FAO, 1983) and the World Health Organization (WHO, 1998) have provided recommendations concerning the risk for the intake of trace elements from food. Safety intakes must be established where there is no risk, as far as it can be judged from the available scientific evidence (COT, 2004). Consequently, suitable analytical control of fresh, processed and canned fish and shellfish is needed so that safe consumption is ensured.

Several analytical techniques are available for trace element determination in seafood samples such as inductively coupled plasma-optical emission spectrometry (ICP-OES)

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(Ikem & Egiebor, 2005), inductively coupled plasma-mass spectrometry (ICP-MS) (Falcó, Llobet, Bocio, & Domingo, 2006), hydride generation-atomic absorption spectrometry (Serafimovski, Karadjova, Stafilov, & Tsalev, 2006), cold vapour-atomic absorption spectrometry (Rio-Segade & Bendicho, 1999), hydride generation-atomic fluorescence spectrometry (Wei, Gupta, Hernández, & Farhat, 1999) and electrothermal-atomic absorption spectrometry (ETAAS) (Sepe et al., 2003). For trace metal determination in fish, matrix usually needs to be removed so that to convert the sample to the liquid state prior to determination. Wet and dry oxidation procedures have been extensively applied, which involve strong reaction conditions such as the use of mineral acids, oxidizing agents and high temperature and pressure conditions (Kwockek, Szefer, Hac, & Grembecka, 2006; Plessi, Bertelli, & Monzani, 2001; Sheppard, Heitkemper, & Gaston, 1994; Tahán, Sánchez, Granadillo, Cubillán, & Romero, 1995; Tuzen & Soylak, 2007; Ybanez, Cervera, & Montoro, 1992).

In recent years, ultrasound-assisted extraction of trace elements from a variety of matrices using both bath and probe ultrasonic processors has emerged as an efficient approach for sample preparation in trace element analysis (Ashley, Andrews, Cavazos, & Demange, 2001; Júnior, Krug, Pereira, & Korn, 2006). Advantages of this technique have been widely recognized for efficient separation of metals and metalloids from a variety of solid matrices, hence avoiding intensive and destructive sample treatments such as dry ashing or acid digestion procedures. Ultrasonic probes provide some advantages in comparison with ultrasonic baths, namely, short extraction times, extraction efficiency approaching 100% and feasibility of using low acidic conditions for extraction as a result of the enhanced ultrasonic power delivered by the former (i.e., high intensity sonication), which can be directly focused onto the sample (Bendicho & Lavilla, 2000).

Several applications have been reported for the use of ultrasonic baths, i.e., the most extended ultrasonic processor, concerning metal extraction from fish samples (El Azouzi, Cervera, & de La Guardia, 1998; Manutsewee, Aeungmaitrepirom, Varanusupakul, & Imyim, 2007). Nevertheless, it should be highlighted that in those applications, long extraction times and acids at high concentration need to be applied in order to achieve efficient extractions. Although several applications have appeared in the literature concerning high intensity ultrasonic extractions of metals from seafood, most of them have dealt with certified reference materials (CRMs), e.g., mussel tissue (Capelo, Lavilla, & Bendicho, 1998; Krishna & Arunachalam, 2004) but the number of applications in seafood control following a systematic approach is almost null.

The aim of this work is to develop a fast and accurate method for determination of As, Se, Ni and V in a variety of fish and shellfish (fresh and frozen products) by electrothermal-atomic absorption spectrometry following ultrasound-assisted extraction as an alternative to dry ashing or wet digestion methods for sample pre-treatment.

2. Material and methods

2.1. Reagents

All chemicals used were of analytical-reagent grade. Ultrapure water was obtained from a Milli-Q™ water system (Millipore, Bedford, MA, USA). HNO_3 (Merck) was used for extraction after suitable dilution. A stock standard solution of Ni (1000 mg/L) was obtained by dissolving the appropriate amount of the pure metal (Merck). An As stock standard solution (1000 mg/L) was prepared from As_2O_3 (Merck). A 1000 mg/L Se stock standard solution (H_2SeO_3 in 1 mol/L HNO_3) was provided by Panreac. A stock standard solution of V (1000 mg/L) in 1% v/v HNO_3 was obtained from Fluka.

Calibration standards were prepared by suitable dilution of these stock standard solutions in 0.5% v/v HNO_3. $Pd(NO_3)_2$ and $Mg(NO_3)_2$ (Merck) were used as matrix modifiers for As and Se determination by ETAAS. The following certified reference materials (CRMs) were used for validation purpose: NRCC-DORM-2 dogfish muscle, NRCC-DOLT-2 dogfish liver, NRCC-TORT-2 lobster hepatopancreas, BCR 627 tuna fish and NIST-SRM 1566b oyster tissue.

2.2. Apparatus

A Unicam Solaar 939 atomic absorption spectrometer (Cambridge, UK) equipped with deuterium background corrector was employed in combination with an Unicam GF-90 graphite furnace and a Unicam FS 90 autosampler for Ni and V determination. Hollow cathode lamps of Ni and V were employed as radiation sources. A Perkin–Elmer (Uberlingen, Germany) Model 4110 ZL atomic absorption spectrometer equipped with longitudinal Zeeman background correction was employed in combination with a Perkin–Elmer AS-72 autosampler for the determination of As and Se. Electrodeless discharge lamps of As and Se (EDL system II, Perkin–Elmer) were used as radiation sources. The instrumental parameters are shown in Table 1.

Table 1
Instrumental parameters for determination of As, Se, Ni and V by electrothermal-atomic absorption spectrometry

Parameter	As	Se	Ni	V
Wavelength/nm	193.7	196.0	232.0	318.5
Spectral bandpass/nm	0.7	2.0	0.2	0.7
Background correction	Zeeman	Zeeman	D_2	D_2
Radiation source[a]	EDL	EDL	HCL	HCL
Lamp current/mA	380	380	10	15
Atomizer type[b]	THGT	THGT	PCGT	PCGT
Signal measurement[c]	PA	PA	PA	PA
Read time/s	5	6	3	3

[a] EDL: electrodeless discharge lamp; HCL: hollow cathode lamp.
[b] THGT: transverse heated graphite graphite tube with integrated L'vov platform; PCGT: pyrolytically-coated graphite tube.
[c] Peak area.

A 100 W 20 KHz VC Sonics and Materials high intensity ultrasonic processor (Danbury, CT, USA) equipped with a 3 mm diameter titanium microtip was employed for ultrasound-assisted extraction. A microbalance MC5 (Sartorius, Germany) with 1 μg readability was used for weighing samples. A Retsch (Haan, Germany) mixer mill MM 2000 equipped with 10 mL capacity agate cups and agate balls were used for grinding the fish and shellfish samples once they were dried. Sieves made of nylon for selecting fractions with particle size of <25, <50, <100 and <200 μm were used.

Optimized thermal programs for As, Se, Ni and V are shown in Table 2. Atomization conditions were established using both sonicated extracts from mussel tissue and aqueous standards prepared in 3% v/v HNO_3 solution.

Despite several matrix modifiers being available for determination of Ni and V in biological matrices [e.g. ammonium phosphate, the mixture $Pd(NO_3)_2 + Mg(NO_3)_2$], these elements can be successfully determined without matrix modifier. A 10 μL of standard/sample volume and no matrix modifier were employed for determination of Ni and V. Standard/sample (5 μL) and 15 μL of matrix modifier containing 22.5 μg Pd + 13.5 μg $Mg(NO_3)_2$ for As determination or 22.5 μg Pd for Se determination, were used.

2.3. Sample pre-treatment for fish and shellfish

The analyzed samples were: hake (*Merluccius merluccius*), sole (*Solea solea*), clam (*Venerupis rhomboides*), prawn (*Panaeus kerathurus*), cuttlefish (*Sepia officinalis*), shrimp (*Palaemon elegans*), razor shell (*Ensis ensis*), cockle (*Cardium edule*), Mussel (*Mytilus galloprovincialis*), edible crab (*Cancer pagurus*), meagrin (*Lepidorhombus whiffiagonis*). These samples were purchased in local markets being representative of what is commonly consumed in this region (Galicia, Spain). Only the commonly edible part of the animal was selected for analysis. Once in the laboratory, the samples were homogenized using a mixer and then, freeze-dried (−48 °C; 5.8×10^{-2} mB) and ground with the vibrational agate ball-mill. The powdered samples were sieved to obtain fractions with different particle size. Samples (50 g) were stored in closed polyethylene tubes and kept at 4 °C until analysis.

Table 2
Thermal programs for determination of As, Se, Ni and V by electrothermal-atomic absorption spectrometry following ultrasound-assisted extraction

Stage	Temperature (°C)	Hold time (s)	Ramp (s)	Gas flow-rate (mL/min)
Arsenic				
Drying 1	110	30	1	250
Drying 2	130	30	15	250
Pyrolisis	1200	20	10	250
Atomisation	2100	5	0	0[a]
Cleaning	2450	3	1	250
Selenium				
Drying 1	110	30	1	250
Drying 2	130	30	15	250
Pyrolisis	1300	20	10	250
Atomisation	2200	6	0	0[a]
Cleaning	2450	3	1	250
Nickel				
Drying 1	120	40	10	250
Drying 2	300	12	10	250
Pyrolisis	1200	12	100	250
Atomisation	2500	4	0	0[a]
Cleaning	2700	3	100	250
Vanadium				
Drying 1	120	40	10	250
Drying 2	300	12	10	250
Pyrolisis	1200	12	100	250
Atomisation	2700	4	0	0[a]
Cleaning	2800	3	100	250

[a] Read.

2.4. Ultrasonic extraction of As, Se, Ni and V from fish

The fraction with particle size less than 100 μm was selected for analysis. For As, Ni and V, a portion of 10 mg of the powdered samples was weighed in polystyrene vials (2 mL capacity) and 1.5 mL of diluted nitric acid solution (3% v/v) was added. For Se, a portion of 10 mg of the powdered samples and 1.5 mL of 0.5% v/v HNO_3 were used. The suspended sample was sonicated for 3 min at 50% vibrational amplitude of the probe and placed into the autosampler tray. The disrupted solid particles undergone a very fast settling so that centrifugation was unnecessary. Determination of As, Se, Ni and V were carried out in the supernatant using calibration with aqueous standards.

3. Results and discussion

3.1. Optimization of ultrasound-assisted extraction of As, Se, Ni and V

In order to establish optimum extraction conditions, the Plackett–Burman (P–B) fractional factorial design was used. For a number of variables to be evaluated between 4 and 8, the P–B matrix containing eight experiments is suitable (Araujo & Brereton, 1996).

The upper (+) and lower (−) levels for each variable were chosen according to previous experiences. Variables studied and levels (+) and (−) were set up as follows: (A) HNO_3 concentration (% v/v) [level (+): 3; level (−): 0.5]; (B) sample mass (mg) [level (+): 20; level (−): (10); (C) Amplitude of the probe vibration (%) [level (+): 50; level (−): 20]; (D) Particle size (μm) [level (+): fraction with particle size between 100 and 200 μm; level (−): fraction with particle size less than 50 (μm)]; (E) extraction time (min) [level (+): 3; level (−): 1]. The programmed experiments were randomly performed and are shown in Table 3. Result of each experiment was the average value of three separate ultrasound-assisted extractions. Mussel tissue (*Mytilus galloprovincialis*) was used as target sample for optimization purposes.

Table 3
Plackett–Burman experimental matrix for five variables

Experiment	Variable[a]					Experiment order	Results
	A	*B*	*C*	*D*	*E*		
1	+	+	+	−	+	(8)	y_1
2	+	+	−	+	−	(5)	y_2
3	+	−	+	−	−	(4)	y_3
4	−	+	−	−	+	(7)	y_4
5	+	−	−	+	+	(3)	y_5
6	−	−	+	+	+	(2)	y_6
7	−	+	+	+	−	(6)	y_7
8	−	−	−	−	−	(1)	y_8

[a] Variable *A*: HNO_3 concentration (% v/v); variable *B*: sample mass (mg); variable *C*: amplitude of the probe vibration (%); variable *D*: particle size (μm); variable *E*: extraction time (min).

The main effect of a variable (e.g., variable *A*) was estimated as follows:

$$\text{Main effect } (A) = \frac{(y_1 + y_2 + y_3 + y_5)}{4} - \frac{(y_4 + y_6 + y_7 + y_8)}{4}$$

where y_i is the result of each experiment (μg/g). Main effects were considered to be significant when they were twice the average standard deviation from all experiments ($2\bar{s}$). Standardized effects for As, Se, Ni and V are shown in Fig. 1.

3.2. Effect of the nitric acid concentration in the extractant

The presence of an acidic medium at low concentration facilitates extraction because when combined with the ultrasonic action makes the solid particles more flocculent. In this study, HNO_3 concentration was fixed at 0.5% and 3% v/v. No effect was seen for Ni and V, but a significant effect was seen for As and Se. Whereas the effect is positive for As, a negative effect was observed for Se. This fact is attributed to the readsorption phenomenon that occurs for Se when the highest level of this variable is employed (i.e., 3% v/v HNO_3). Therefore, As, Ni and V could be extracted in 3% v/v HNO_3 but a different extraction needs to be performed for Se using an 0.5% v/v HNO_3 extractant.

3.3. Effect of the sample mass

Low sample masses could give rise to poor precision owing to increased influence of inhomogeneity with decreasing mass in suspension. On the other hand, too concentrated suspensions could give rise to particle agglomeration and decreased ultrasonic action. Negative effects of this variable can be observed for As, Se and V. Non-significant effect occurs for Ni. Consequently, the lowest level of this variable (i.e., 10 mg) should be employed.

3.4. Effect of the particle size

This variable should influence to some extent, but the disruption action caused by ultrasound could counteract the negative effect caused by increasing particle size. Only a negative effect was observed for Ni extraction. When this variable was studied for Ni using an univariate approach using the fractions (Ø, particle size): $Ø < 25$; $Ø < 50$; $Ø < 100$ and $100 < Ø < 200$ μm, no influence was observed for a particle size less than 100 μm. For convenience, the fraction with particle size less than 100 μm can be used for efficient extraction in all cases.

3.5. Effect of the sonication amplitude

The energy delivered by ultrasound to the liquid medium depends on the amplitude of the probe vibration. Extraction efficiency should increase with increasing ultrasound amplitude. Whereas no effect is seen for V and Ni, positive effects occur for As and Se. The highest level screened of this variable (i.e., 50%) needs to be employed for efficient extraction of the four elements.

3.6. Effect of the sonication time

Applying probe sonication instead of bath sonication can drastically shorten the time needed for metal extraction from powdered samples in suspension. Despite non-signif-

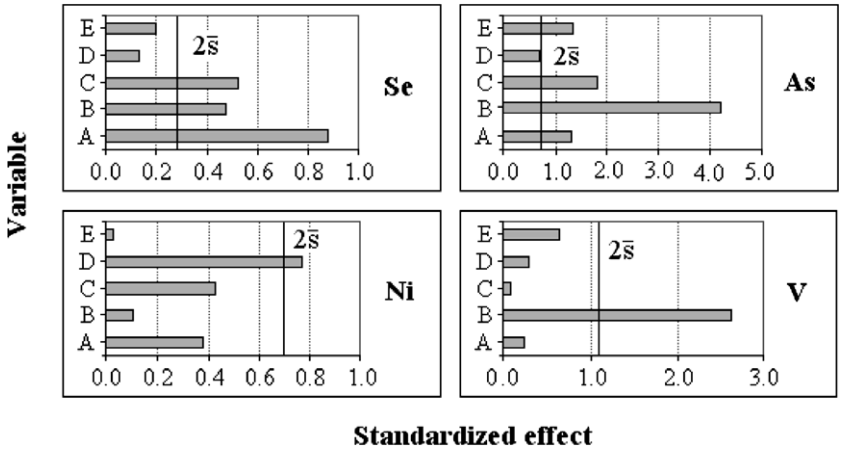

Fig. 1. Standardized effects (μg/g) for the variables influencing the ultrasound-assisted extraction of As, Se, Ni and V from mussel tissue. The vertical lines marked represent the experimental error ($2\bar{s}$).

icant effects being observed for Ni, V and Se, the maximum value of this variable (i.e., 3 min) was used for efficient extraction of the four elements.

When considering the effect of all variables, it can be concluded that same extraction conditions can be employed for As, Ni and V. On the contrary, Se needs to be extracted in a separate run using a lower nitric acid concentration.

3.7. Analytical characteristics

Analytical characteristics were obtained for the four elements studied under optimized conditions.

Calibration curves were linear at least up to 2000 ($R^2 = 0.995$), 1000 ($R^2 = 0.994$), 50 ($R^2 = 0.998$) and 100 ($R^2 = 0.999$) ng/mL for As, Se, Ni and V, respectively. Characteristics masses were also estimated, being in good agreement with the recommended values provided by the manufacturer. Experimental values for the characteristic mass (pg) were 28.6, 61, 10.5 and 12.6, for As, Se, Ni and V, respectively. Limits of detection calculated according to the 3s criterion were 0.6, 0.3, 0.2 and 0.4 μg/g for As, Se, Ni and V, respectively. For establishing between-batch precision values, three subsamples were subjected to the extraction procedure. Between-batch precision expressed as relative standard deviation were in the ranges 2.7–4.8% for As, 4.2–11% for Se, 3.0–8.4% for Ni and 2.7–10% for V.

3.8. Validation

The method has been validated against five CRMs (Table 4). These materials were employed as provided without further grinding. Validation of As was made with DORM-2 and BCR 627 (tuna fish). The latter material was certified for total As, DMA and AsB. Validation of Se was carried out with DORM-2, DOLT-2 and TORT-2. Validation of V and Ni was carried out with TORT-2 and NIST 1566b.

Extractions ($N = 5$) were performed under suitable conditions found after optimization. A t-test was applied for testing the accuracy of the results. The condition $t_{exp} < t_{crit}$ was fulfilled in all cases, and consequently, non-significant differences ($p = 0.05$) were observed between the certified and found metal contents.

3.9. Analysis of seafood samples

Analytical results for the determination of As, Se, Ni and V in 12 seafood samples are shown in Table 5. In all cases, average values ± standard deviation ($N = 3$) are shown. Metal contents were determined on a dry weight basis. As can be observed, metal contents in the samples studied depend on the analyzed species. Thus, contents (μg/g, dry weight) in the samples analyzed were in the range of 12.6–190 for As, 0.73–2.34 for Se, 2.94–46 for Ni and 0.82–5.14 for V.

Marine organisms can accumulate As and convert it into organoarsenicals (Neff, 2002). As toxicity largely depends on the chemical form, exposure to inorganic As being the most harmful. The WHO has established a limit for the safe As (as inorganic forms) intake in 15 μg/kg of body weight. Determination of As in marine biological tissues is troublesome due to the presence of interferences, difficulties in mineralization of some organic As species such as arsenobetaine (i.e., the predominant As species in fish tissues), or As losses during dry oxidation due to volatilization. Determination of As in fish by ETAAS requires Zeeman correction to compensate for the presence of structured background (Santos, Alava-Moreno, Lavilla, & Bendicho, 2000). As concentrations ranged from 12.6 μg/g for mussel to 190 μg/g for prawn.

Selenium is an essential trace element for humans, displaying a narrow boundary between essentiality and toxicity. Se takes part of selenoproteines such as glutathione peroxidase. The RDA has given minimum Se requirements in 70 and 55 μg/day for males and females (RDA, 1989). The lowest and highest Se contents were found as 0.73 μg/g in cockle and 2.34 μg/g in edible crab, respectively.

Vanadium and nickel are present as porphyrins in crude oils. Data concerning V in seafood are scarce, but bioaccumulation of V in molluscs has been suggested (Edel & Sabbioni, 1993). Ni can cause toxicity if its levels exceed the

Table 4
Validation of the ultrasound-assisted extraction method for As, Se, Ni and V

CRM	Element	N	Certified value (μg/g) ($\bar{x} \pm CI$)*	Found value (μg/g) ($\bar{x} \pm CI$)*
NRCC DORM-2 (dogfish muscle)	As	5	18.0 ± 1.1	17.6 ± 0.46
	Se	5	1.40 ± 0.09	1.37 ± 0.10
NRCC DOLT-2 (dogfish liver)	Se	5	6.06 ± 0.49	5.98 ± 0.21
NRCC TORT-2 (lobster hepatopancreas)	Se	5	5.63 ± 0.67	5.76 ± 0.20
	Ni	5	2.50 ± 0.19	2.34 ± 0.18
	V	5	1.64 ± 0.19	1.58 ± 0.13
BCR 627 (tuna fish)	As	5	4.8 ± 0.3	5.1 ± 0.46
NIST SRM 1566b (Oyster tissue)	Ni	5	1.04 ± 0.019	0.97 ± 0.09
	V	5	0.577 ± 0.023	0.61 ± 0.035

* Average value ± confidence interval ($p = 0.05$); $t_{crit} = 2.571$.

Table 5
Analytical results (average value ± standard deviation; $N = 3$) for As, Se, Ni and V in fish and shellfish

Sample	As	Se	Ni	V
Clam (*Venerupis rhomboides*)	18.3 ± 0.8	1.42 ± 0.06	6.20 ± 0.52	2.93 ± 0.03
Cockle (*Cardium edule*)	13.4 ± 0.6	0.73 ± 0.07	46 ± 2	5.14 ± 0.14
Cuttlefish (*Sepia officinalis*)	75.9 ± 2.2	1.56 ± 0.11	7.65 ± 0.47	0.95 ± 0.12
Edible crab (*Cancer pagurus*)	39.3 ± 1.4	2.34 ± 0.15	24.4 ± 0.76	3.54 ± 0.12
Hake (*Merluccius merluccius*)	16.0 ± 0.6	1.07 ± 0.07	7.67 ± 0.34	0.82 ± 0.05
Meagrin (*Lepidorhombus whiffiagonis*)	42.5 ± 1.3	1.10 ± 0.12	3.24 ± 0.15	1.25 ± 0.08
Mussel (*Mytilus galloprovincialis*)	12.6 ± 0.4	2.1 ± 0.09	2.94 ± 0.09	2.73 ± 0.12
Prawn (*Panaeus kerathurus*)	190 ± 8	1.76 ± 0.08	12.5 ± 0.5	2.04 ± 0.12
Razor shell (*Ensis ensis*)	18.1 ± 0.5	1.35 ± 0.1	5.74 ± 0.22	3.75 ± 0.22
Shrimp (*Palaemon elegans*)	48.2 ± 2.3	0.95 ± 0.09	4.9 ± 0.3	2.14 ± 0.16
Sole (*Solea solea*)	12.9 ± 0.4	1.26 ± 0.1	5.17 ± 0.26	1.17 ± 0.06

Metal concentrations expressed as μg/g (dry weight).

regulated values in foods. The WHO recommends 100–300 μg of Ni for daily intake. The lowest and highest V contents were found as 0.82 μg/g in hake and 5.14 μg/g in cockle. For Ni, the minimum content is found in mussel (2.94 μg/g) and the maximum in cockle (46 μg/g).

Contents (μg/g, wet weight) were in the range: 2.5–38 for As, 0.15–0.47 for Se, 0.6–9.2 for Ni and 0.16–1.0 for V. These results found in the analyzed fish and shellfish samples were acceptable for human consumption at nutritional and toxic levels.

4. Conclusions

Ultrasound-assisted extraction has been applied as a fast, accurate and simple method for establishing the contents of As, Se, Ni and V in fish and shellfish in combination with ETAAS. As compared with acid digestion, faster, safer and less expensive sample pre-treatment can be accomplished with minimum acid consumption. When high intensity ultrasonic processors are employed instead of ultrasonic baths, a low acidic extractant (3% v/v HNO_3) and a short extraction time (3 min) are feasible. This methodology should facilitate the assessment of metal pollutants in seafood on a routine basis. The implementation of multi-probe systems can offer further improvements, as simultaneous high intensity sonications would enhance the sample throughput.

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