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In vitro bioavailability of total selenium and selenium species from seafood

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ABSTRACT

In vitro bioavailability of total selenium and selenium species from different raw seafood has been assessed by using a simulated gastric and intestinal digestion/dialysis method. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to assess total selenium contents after a microwave assisted acid digestion, and also to quantify total selenium in the dialyzable and non-dialyzable fractions. Selenium speciation in the dialyzates was assessed by high performance liquid chromatography (HPLC) coupled with ICP-MS detection. Major Se species (selenium methionine and oxidized selenium methionine) from dialyzate were identified and characterized by HPLC coupled to mass spectrometry (HPLC–MS). Selenocystine was detected at low concentrations while Se-(Methyl)selenocysteine and inorganic selenium species (selenite and selenate) were not detected in the dialyzate. Low bioavailability percentages for total selenium (6.69 ± 3.39 and 5.45 ± 2.44% for fish and mollusk samples, respectively) were obtained. Similar bioavailability percentages was achieved for total selenium as a sum of selenium species (selenocystine plus oxidized selenium methionine and selenium methionine, mainly). HPLC–MS data confirmed SeMet oxidation during the *in vitro* procedure.

1. Introduction

Selenium has been largely recognized as a nutritionally essential element for human life. This is because selenium (mainly the selenocystine species) is a component of certain enzymes (seleno-enzymes) which play important roles in mammals (Gladyshev & Hatfield, 1999). However, the content of selenium in foodstuff in several regions of the world is insufficient to offer the proper protective activity, and this justifies the growing interest in the production of selenium fortified foods and selenium-based nutritional supplements (functional foods) (Finley, 2005; Schrauzer, 2001). The recommended intake of selenium is different in each country and depends on factors like age and sex. However, 55 μg/day is generally regarded as the appropriate amount (NAH, 2000). Brazil nuts, mushrooms and certain vegetables, such as broccoli, contain high levels of selenium, mainly as selenomethionine (SeMet) (Vonderheide et al., 2002). Other foodstuff such as meat (Shi & Spallholz, 1994), seafood and seaweed are able to concentrate selenium (Chapman & Chapman, 1980).

It has been well established that the total content of an essential element present in a foodstuff is not totally available, i.e., it is not totally assimilated by the human body. Bio-availability of essential and toxic elements from foodstuff can be performed by *in vivo* (dosing experimental animals with several concentrations of the target element) or by *in vitro* methods. Testing with animals is expensive, difficult to perform, and ethically controversial, and it provides limited data in each experiment (Hansen, Sandstrom, & Lonnerdal, 1996). Alternative *in vitro* approaches for assessing bio-availability studies are therefore more advantageous (Intawongse & Dean, 2006). Two different *in vitro* experimental approaches can be performed in bio-availability studies. The simplest method, commonly referred as bio-accessibility, indicates the maximum fraction of a substance (trace element) in food that can theoretically be released from the foodstuff in the gastrointestinal (GI) tract (bio-accessible fraction), and becomes then available for intestinal absorption (i.e. enters into the blood stream) (Oomen et al., 2002). A second approach, formally called bio-availability, refers to the fraction of a substance that reaches the systemic circulation (blood) from the GI tract (bio-available fraction), and it is available to promote its action in the exposed organism (Ruby et al., 1999). Experimental differences between both approaches are mainly focused on simulating intestinal

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absorption by means of dialysis membranes during the simulated intestinal digestion in the bio-availability studies (dialysability approach). It must be mentioned that both bio-availability and bio-accessibility tests are affected by the type of food, the composition of food, and also, by the simulated GI conditions used when performing the experiments (Intawongse & Dean, 2006).

Despite the great deal of literature regarding with the assessment of the bio-accessible fraction of essential and toxic elements and organometallic species in foods, data on the bio-available fraction of those targets (including selenium species) is scarce (Intawongse & Dean, 2006; Moreda-Piñeiro et al., 2011). Specifically, selenium speciation studies in the bio-available fraction (dialyzability approach) from seafood are not reported. However, different studies based on bio-accessibility have been addressed for selenium and selenium species in foodstuff containing high selenium concentrations, such as Brazilian nuts (Dumont, De Pauw, Vanhaecke, & Cornelis, 2006). In addition, some studies have been performed in fish, such as swordfish, sardine, tuna and cod (Cabañero, Madrid, & Cámara, 2004, 2007; Crews et al., 1996), in selenium-fortified foodstuff, such as radish (Pedrero, Madrid, & Cámara, 2006), garlic (Dumont, Ogra, Vanhaecke, Suzuki, & Cornelis, 2006), green onion and chives (Kápolna & Fodor, 2007), yeast and yeast-based nutritional supplements (Dumont, Vanhaecke, & Cornelis, 2004; Hinojosa-Reyes, Marchante-Gayón, García-Alonso, & Sanz-Medel, 2006; Hinojosa-Reyes, Ruiz-Encinar, Marchante-Gayón, García-Alonso, & Sanz-Medel, 2006), wheat (Govasmark et al., 2010), chicken eggs and chicken meat (Lipiec, Siara, Bierla, Ouerdane, & Szpunar, 2010).

The objectives of the current work have been the development of an *in vitro* procedure based on dialysability for assessing the bio-availability of total selenium and selenium species from seafood (fish and mollusk). As previously mentioned, bio-availability (dialyzability) studies for total selenium and selenium species from seafood have not yet been addressed.

2. Materials and methods

2.1. Apparatus

Dionex HPLC UltiMate 3000 LC (Dionex, Sunnyvale, CA, USA), equipped with a GP50 gradient pump (Dionex), an AS50 thermal compartment (Dionex) and an AS50 auto-sampler (Dionex). Thermo Finnigan X Series inductively coupled plasma-mass spectrometer (ICP-MS) from Thermo Fisher Scientific Inc., (Waltham, MA, USA). An Accela binary pump (Thermo Scientific) coupled with an Accela autosampler (Thermo Scientific) and an LTQ-Orbitrap Discovey mass spectrometer (Thermo) was used for selenium species identification and characterization. Targets separation by HPLC–ICP-MS experiments was performed by using a 250 × 4.1 mm i.d. Hamilton PRP X-100 anion exchange column with a guard column (25 mm, 2.3 mm i.d. from Hamilton (Reno, NV, USA). HPLC–MS measurements were carried out with a 150 mm × 4.6 mm i.d., 5.0 μm Phenomenex Luna C18(2) reverse phase column from Phenomenex (Torrance, CA, USA). Lab Blender Stomacher 400 (Seward Med. Ltd., London, UK) with Stomacher closure bags 6041/CLR (Seward). LYPH-LOCK 6 litre freeze dry system, model 77530 from Labconco Corporation (Kansas City, MO, USA). Thermostatic oven, model 207, from Selecta (Barcelona, Spain). Vibrating ball mill from Retsch (Haan, Germany) equipped with zircon cups (15 mL) and zircon balls (7 mm diameter). Ethos Plus microwave lab-station (Milestone, Sorisole, Italy) with 100 mL closed Teflon vessels and Teflon covers, HTC adapter plate and HTC safety springs (Milestone). Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (Selecta). Cellu Sep® H1 high grade regenerated cellulose tubular membranes (molecular weight cut-off 10 kDa, 50 cm length, diameter dry 25.5 mm and a volume to length ratio of 5.10 mL cm^{-1}) were from Membrane Filtration Products Inc. (Texas, USA). ORION 720A plus pH-meter with a glass–calomel electrode (ORION, Cambridge, UK). Cellulose acetate syringe filters (0.45 μm) were from Millipore (MA, USA).

2.2. Reagents

Ultra-pure water of resistance 18 MΩ cm^{-1} was obtained from a Milli-Q purification device (Millipore Co.). Methanol (gradient grade) and formic acid (98–100%) were from Merck (Poole, UK). Citric acid 99% (m/m), ammonia 25% (m/v) and selenite (Se(IV)) stock standard solution, 1000 mg L^{-1}, were from Panreac (Barcelona, Spain). Standard solutions of selenate (Se(VI)), $SeCys_2$, SeMeCys and SeMet (1000 g L^{-1}) were prepared by dissolving the appropriate amounts of sodium selenate (Na_2SeO_4) and SeMeCys (Se-(Methyl)selenocysteine hydrochloride 95%) from Aldrich (Milwaukee, WI, USA); and $SeCys_2$ (seleno-L-cystine 95%) and SeMet (seleno-D,L-methionine 99%) from Sigma Chemicals (St Louis, MO, USA). Digestive enzymes (porcine pepsin, p-7000, porcine pancreatin, P-1750), bile salts (approx. 50% sodium cholate and 50% sodium deoxycholate) and piperazine-NN-bis(2-ethane-sulfonic acid) di-sodium salt (PIPES), were obtained from Sigma Chemicals. Sodium hydrogen carbonate was from Merck. AnalaR nitric acid 69% (m/m), hydrochloric acid 37% (m/m) and hydrogen peroxide 33% (m/v) were from Panreac. DORM-2 (dog–fish muscle) certified reference material (CRM) was from the National Research Council of Canada (Ottawa, Canada).

2.3. Seafood samples

Mollusk, white fish and cold water fish samples were obtained from a local supermarket. Samples were pre-treated as was indicated in a previous paper (Moreda-Piñeiro et al., 2012). All samples were preserved in pre-cleaned polyethylene bottles.

2.4. Microwave assisted acid digestion of samples and dialyzate extracts

Powdered samples (0.5 g) and dialyzates (1.0 mL) were subjected to a microwave assisted acid digestion procedure in triplicate under optimized conditions reported elsewhere (Moreda-Piñeiro et al., 2012). Before ICP-MS measurements, acid digests were filtered through 0.45 μm cellulose acetate syringe filters.

2.5. In vitro digestion and dialysis procedure

The *in vitro* digestion was performed in triplicate by weighing 0.5 g of powdered samples into 100 mL Erlemeyer flasks according to the procedure described elsewhere (Moreda-Piñeiro et al., 2012). Reagent blanks were also obtained to control possible contamination. Dialyzates (~17 mL) were freeze dried and then dissolved in 5.0 mL of mobile phase (20.0 mM of citric acid plus 2.0% (v/v) methanol (HPLC–ICP-MS). The freeze dried residue was however dissolved with 5.0 mL of 1.0% (v/v) formic acid solution plus 10% (v/v) methanol when performing HPLC–MS experiments. Both dialyzate and the residual fractions were kept at −20 °C before measurements.

2.6. ICP-MS measurements

Total selenium in the acid digests and dialyzates was measured by ICP-MS under operating conditions listed in Supplementary Table S1. Determinations were performed by using aqueous standard solutions in 2.0 M nitric acid covering selenium concentrations from 0 to 1000 μg L^{-1}. Germanium at a concentration of

Table 1
Mean slopes for calibration, and limits of detection and quantification (based on the 3 SD and 10 SD criterion of background signal).

	Mean calibration slope ± SD[a] ($L\ \mu g^{-1}$)	LOD[b] ($ng\ g^{-1}$)	LOQ[b] ($ng\ g^{-1}$)
$SeCys_2$	6633 ± 700	2.7	9.0
SeMeCys	5910 ± 630	2.5	8.2
Se(IV)	7200 ± 463	2.9	9.5
SeMet	8950 ± 906	2.7	9.0
Se(VI)	7650 ± 500	3.0	10.0

[a] $n = 8$.
[b] $n = 11$.

10 $\mu g\ L^{-1}$ was selected as an internal standard. ICP-MS determinations gave a limit of detection (LOD) and a limit of quantification (LOQ), based on the 3 SD/10 SD criterion (S.D. standard deviation of eleven measurements of a reagent blank), of 5.0 and 12.0 $ng\ g^{-1}$, respectively. Accuracy of the method was assessed by analyzing DORM-2 (dog–fish muscle) in triplicate. A value of 1.35 ± 0.02 $\mu g\ g^{-1}$ was found; which are in good agreement with the certified value (1.40 ± 0.09 $\mu g\ g^{-1}$).

2.7. HPLC–ICP-MS measurements

Anion exchange HPLC conditions, summarized in Supplementary Table S1, were used to obtain the separation of selenium species (Se(IV), Se(VI), $SeCys_2$, SeMeCys and SeMet). Different calibration curves using germanium (10 $\mu g\ L^{-1}$) as an internal standard were obtained by covering Se(IV), Se(VI), $SeCys_2$, SeMeCys and SeMet concentrations of 0, 0.5, 2.5, 5.0, 10, 20 and 40 $\mu g\ L^{-1}$ (concentrations expressed as selenium). Table 1 lists the mean and the standard deviation of the slopes of calibration graphs for each analyte. A good repeatability of the calibration curves can be seen over six different days, with RSD values around 13% for all cases. Similarly, the LODs and LOQs, expressed as $ng\ g^{-1}$ in accordance with sample mass weight and final volumes, are also listed in Table 1.

2.8. HPLC–Orbitrap MS measurements

Target separation (injection volume of 10 μL) was carried out with the Phenomenex Luna C18(2) coupled to an Orbitrap mass spectrometer for detection. Column temperature was set at 25 °C, and separation was performed in isocratic mode at a flow rate of 0.500 mL min^{-1}. Mobile phase consisted of a formic acid solution (1.0% v/v) plus 10% (v/v) methanol. Under these conditions, $SeCys_2$, SeOMet, SeMeCys and SeMet elute at 2.9, 3.0, 3.8 and 5.2 min, respectively. Detection was performed in positive ion atmospheric pressure chemical ionization (PI-APCI) mode. Ultrahigh-purity nitrogen (99.999%) was used as the sheath (50 arbitrary units) and auxiliary gas (10 arbitrary units). The analyzer was FTMS with a scan range of m/z 50.00–600.00 (resolution 30,000). Capillary temperature was set at 350 °C, tube lens were set at 99.9 V, and capillary voltage at 47.9 V. $SeCys_2$, SeOMet, SeMeCys and SeMet are charged molecules and their identification was based on $[M+H]^+$ of 336.9204, 215.0115, 183.9870 and 198.0028, respectively. Aqueous standards and dialyzates were matched with mobile phase (formic acid solution (1.0% v/v) plus 10% (v/v) methanol).

3. Results and discussion

3.1. Total selenium in fish and mollusk

Total selenium ranged from 470 to 950 $ng\ g^{-1}$ (dry weight (d)) in mollusk, whereas, concentrations within the 330–650 $ng\ g^{-1}$ (dry weight (d)) range were determined in fish samples (Table 2). These concentration values agree with selenium data reported in the literature. As examples, selenium levels of 1520 ± 70 $ng\ g^{-1}$ (wet weight (w)) (Crews et al., 1996); and 250 ± 20, 380 ± 20 and 500 ± 20 $ng\ gg^{-1}$ (w) (Cabañero et al., 2004, 2007) for uncooked fish tissues were reported. Selenium values within the 67–734 $ng\ g^{-1}$ (w) range were also reported by Plessi, Bertelli, and Monzani (2001) in different seafood types. Similarly, Lavilla, Vilas, and Bendicho (2008) reported selenium values from 730 to 2340 $ng\ g^{-1}$ (d) in mollusk and fish; while Afonso et al. (2008) have given selenium concentrations within the 360–460 $ng\ g^{-1}$ (w) range in several fish.

3.2. Bio-available total Se and Se species in fish and mollusk

The bio-availability, expressed as a percentage, was calculated using the following equation:

$$\text{Bav}\ (\%) = \frac{[Se]_{\text{dialyzate extract}}}{[Se]_{\text{acid digest}}} \times 100$$

where Bav (%) is the percentage of bio-availability, and $[Se]_{\text{dialyzate extract}}$ and $[Se]_{\text{acid digest}}$ are the selenium concentrations after *in vitro* digestion and after the microwave assisted acid digestion procedures, respectively.

As shown in Table 2, the bio-available total selenium concentrations, referred to the mean of three assays for each sample, were within the 19–78 $ng\ g^{-1}$ range for fish and mollusk. Fig. 1 shows the bio-availability percentages of total selenium and total selenium (as a sum of selenium species concentrations) in white fish, cold water fish and mollusks. The bio-availability of selenium in white fish, cold water fish and mollusk was ranged from 4.0% to 13%. The bio-availability percentages obtained in the current investigation are lower than those published data regarding bio-accessible total selenium from seafood, i.e. percentages from 50% to 83% from cod, tuna, swordfish and sardine (Cabañero et al., 2004, 2007; Crews et al., 1996). These differences are attributed to the additional dialysis stage added in the current *in vitro* bio-availability method, which improves the simulation of the intestinal digestion (a dialyzability stage is included in the simulation process), and lead therefore to lower bio-available percentages for total selenium. It can also be concluded that total selenium is highly bio-accessible (Cabañero et al., 2004, 2007; Crews et al., 1996) but it is not easily dialysed (bio-available) from fish and mollusk.

On the other hand, the bio-available selenium species concentrations from fish and mollusk, expressed as $ng\ g^{-1}$, was within the 9–23 range for $SeCys_2$ plus SeOMet (see next section) and within the <9–58 $ng\ g^{-1}$ range for SeMet (Table 2). Levels of dialyzable SeMeCys, Se(IV) and Se(VI) were lower than the LOQs of the method for these species (8, 10 and 10 $ng\ g^{-1}$, respectively) for all analyzed samples. As can be seen the major species in the fish and mollusks are SeMet and $SeCys_2$ + SeOMet. Furthermore, total selenium (as sum of selenium species) in the dialyzate fraction measured by HPLC–ICP-MS in each sample (Table 2) relates closely to total bio-available selenium in the dialyzate fraction directly measured by ICP-MS (Table 2).

3.3. Se species identification and characterization

A common source of error in selenium speciation studies is attributed to the occurrence of oxidized SeMet. Part of SeMet appears as a separate oxidized selenium methionine (SeOMet) chromatographic peak, leading to the underestimation of SeMet concentration (Pedrero, Ruiz Encinar, Madrid, & Camara, 2007). The oxidation of SeMet after *in vitro* procedures or enzymolysis

Table 2
Total selenium concentrations in seafood after microwave – assisted acid digestion and ICP-MS determination (MAD/ICP-MS) and after the *in vitro* approach and ICP-MS determination (*in vitro* dialysis – ICP-MS); and selenium species concentrations ($SeCys_2$ + SeOMet and SeMet) in seafood after the *in vitro* approach and HPLC–ICP-MS determination.

Sample	Se species concentration[a] (ng g^{-1})				
	MAD/ICP-MS	*In vitro* dialysis – ICP-MS	$SeCys_2$ + SeOMet[b]	SeMet	Total Se (as sum of species)
Edible cockle (*Cerastoderma edule*)	560 ± 30	31 ± 1	14 ± 1	10 ± 1	24 ± 1
Razor shell (*Ensis ensis*)	910 ± 40	78 ± 2	11 ± 1	67 ± 3	78 ± 3
Variegated scallop (*Chlamys varia*)	950 ± 30	24 ± 1	15 ± 1	<9	15 ± 1
Carpet-shell clam (*Tapes decussates*)	830 ± 20	33 ± 1	23 ± 3	11 ± 1	34 ± 3
Scallop (Pecten maximus)	470 ± 10	22 ± 2	15 ± 1	<9	15 ± 1
Hake (Merluccius merluccius)	500 ± 10	64 ± 1	22 ± 2	40 ± 2	62 ± 3
Cod (Gadus gadidae)	650 ± 20	51 ± 4	18 ± 1	36 ± 2	54 ± 2
Anglerfish (Lophius piscatorius)	500 ± 10	20 ± 2	9 ± 1	21 ± 2	30 ± 2
Atlantic pomfret (Brama brama)	330 ± 10	21 ± 1	14 ± 1	11 ± 1	25 ± 2
Poor cod (Trisopterus minutes)	430 ± 20	25 ± 2	9 ± 1	10 ± 1	19 ± 1
Tuna (Thunnus thynnus)	360 ± 10	28 ± 1	9 ± 1	19 ± 1	28 ± 2
Sardine (Sardina pilchardus)	490 ± 40	31 ± 1	10 ± 1	20 ± 1	30 ± 1
Atlantic mackerel (Scomber scombrus)	550 ± 20	41 ± 2	10 ± 1	35 ± 2	45 ± 2
Atlantic horse mackerel (Trachurus trachurus)	440 ± 30	19 ± 1	9 ± 1	12 ± 1	21 ± 1

SeMeCys concentration was under LOQ (8 ng g^{-1}).
Se(IV) and Se(VI) concentration were under LOQ (10 ng g^{-1}).
[a] $n = 3$.
[b] LOQ of $SeCys_2$ + SeOMet = 9 ng g^{-1}.

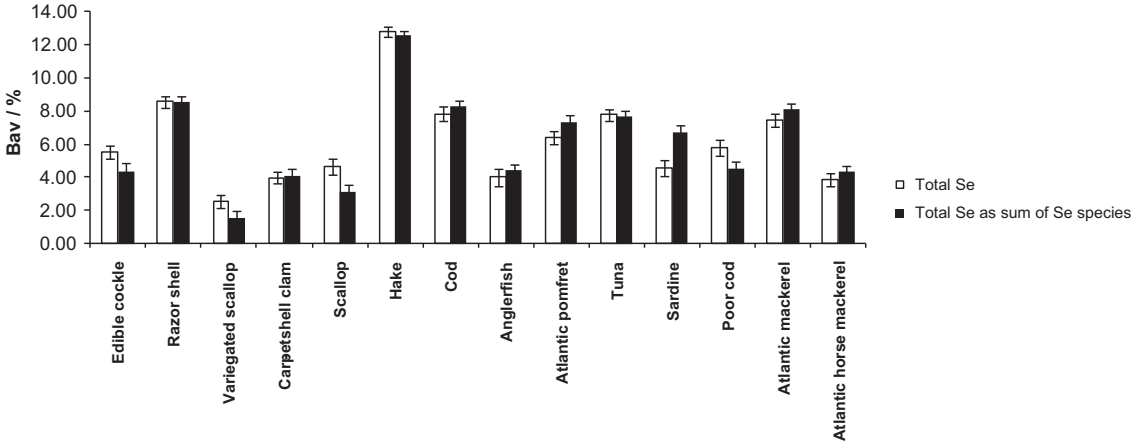

Fig. 1. Bioavailability of total selenium in seafood determined using an *in vitro* method.

procedures has been previously reported (Dumont et al., 2004). On the other hand, when further Se species separation is performed by using Hamilton PRP-X100 column, the eluted selenium species (chromatographic peaks around 2.0–5.0 min) can be attributed to both $SeCys_2$ and SeOMet species (Mazej, Falnoga, Veber, & Stibilj, 2006).

Thus, Orbitrap mass spectrometry was used to confirm the presence of some organoselenium species ($SeCys_2$, SeMet and SeOMet) in dialyzates from the razor shell sample. The identification was based on $[M+H]^+$ at m/z ratios of 336.9204 and 198.0028 which were obtained by injecting $SeCys_2$ and SeMet standards. For SeOMet, the identification was based on $[M+H]^+$ at a *m/z* ratio of 215.0115 by injecting a dialyzate extract. Fig. 2a–c shows the ESI full MS (50.00–600.00), as well as the extracted chromatograms at *m/z* ratios of 336.9204 ($SeCys_2$, Fig. 2a), 198.0028 (SeMet, Fig. 2b) and 215.0115 (SeOMet, Fig. 2c). The mass spectra from each chromatographic peak show the presence of $SeCys_2$, SeMet and SeOMet in the dialyzate from the razor shell sample. This fact is in accordance with the detection/quantification of $SeCys_2$ and SeMet species in this sample by HPLC–ICP-MS. Finally, the presence of SeOMet in the dialyzate is also confirmed, which lead to the overestimation of $SeCys_2$ concentration by HPLC–ICP-MS when a Hamilton X-100 column is used.

3.4. Mass balance

To assess the accuracy of the current bio-availability study, a mass-balance approach was performed by using the Atlantic horse mackerel sample as a model sample. After the *in vitro* procedure (Section 2.5), total selenium and selenium species ($SeCys_2$ + SeOMet, SeMeCys, SeMet, Se(IV), and Se(VI)) concentrations were determined in the dialyzates (Sections 2.6 and 2.7, respectively), while the residual fraction from the *in vitro* method was acid-digested in triplicate (Section 2.4) and analyzed then for total selenium (Section 2.6).

As previously mentioned, Table 2 lists the total selenium concentrations in the dialyzate (*in vitro* digestion and direct ICP-MS determination) in the Atlantic horse mackerel, and also the total selenium concentration in this sample after microwave assisted acid digestion and ICP-MS determination (0.019 ± 0.0010 and 0.44 ± 0.018 μg g^{-1}, respectively). The determination of total selenium in the residual fraction from the *in vitro* method gave a concentration for total selenium of 0.39 ± 0.025 μg g^{-1}. Therefore, a mass balance study was performed by a statistical comparison between the selenium concentration as a sum of total selenium concentrations in the dialyzable and non-dialyzable fractions (0.019 ± 0.0010 plus 0.39 ± 0.025 μg g^{-1}, which gives a total sele-

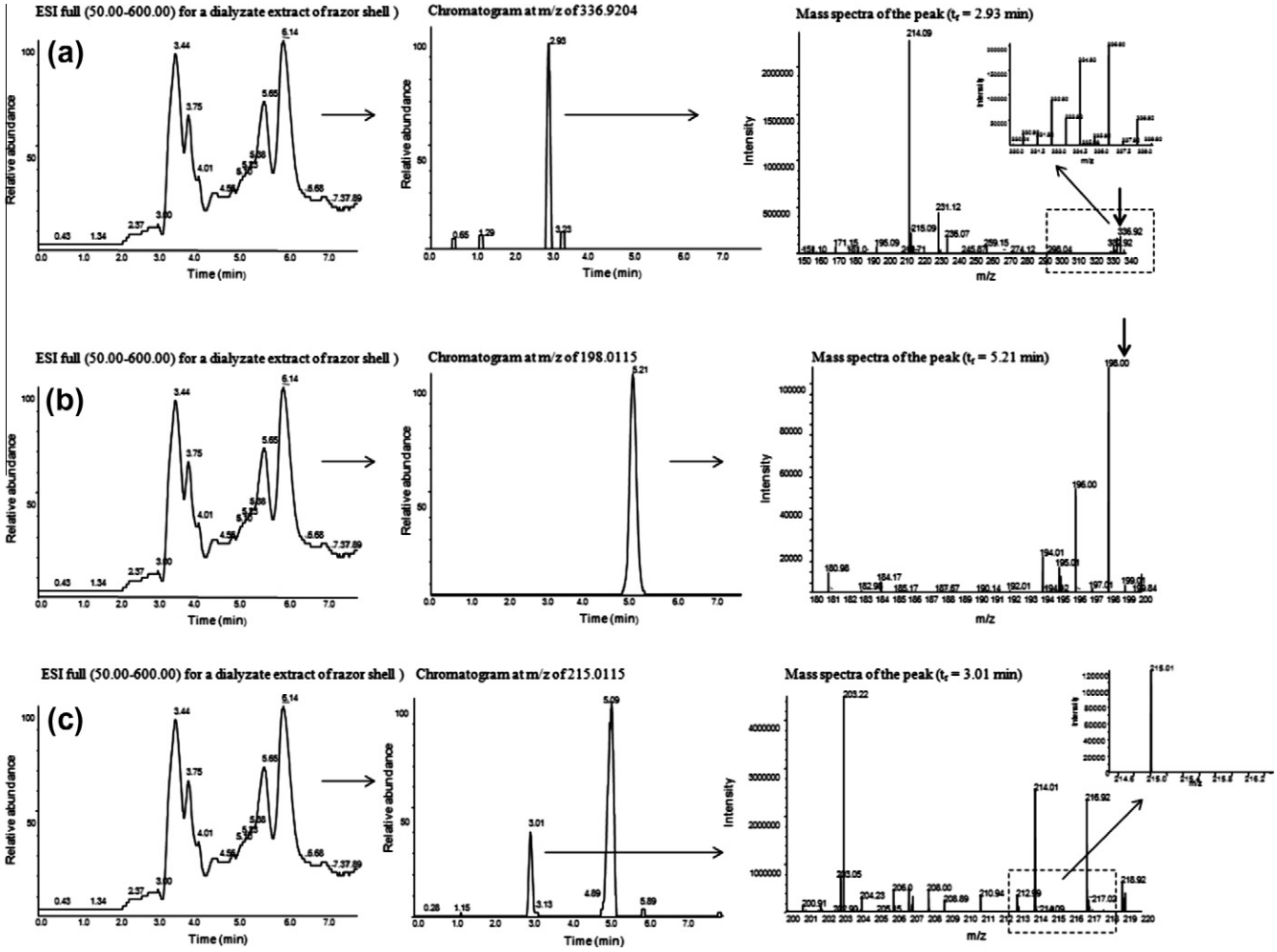

Fig. 2. ESI full MS (50.00–600.00) chromatogram of an dialyzate digest from a razor shell sample. Chromatograms at *m*/z ratios of 336.9204 for $SeCys_2$ (a), 198.0028 for SeMet (b) and 215.0115 for SeOMet (c), and mass spectra of the peak of the extracted chromatogram for $SeCys_2$ (a), SeMet (b) and SeOMet (c).

nium concentration of 0.41 ± 0.025 μg g^{-1}), and the total selenium in the sample (0.44 ± 0.018 μg g^{-1}). Firstly, a statistical comparison of the standard deviation was established by means of the Cochran's C and Bartlett's tests. The *p*-values after Cochran'C and Bartlett's test at a 95% confidence level ($\alpha = 0.05$) for variance check were 0.6828 and 0.6804 respectively. As it can be seen, the smallest of the *p*-values is higher than 0.05 (95.0% confidence level), which indicates that there is not statistically significant difference between the S.D., and an ANOVA test can be performed to compare means. ANOVA results are listed in Supplementary Table S2 where it can be seen that the p-value 0.1669 is higher than 0.05 (95% confidence interval), which means that there are not statistically significant differences between the total selenium concentration, and the sum of selenium concentrations in the dialyzate and in the non-dialyzable fraction. Good accuracy is therefore assessed throughout the *in vitro* procedure for total selenium bio-availability.

Similarly, a statistical evaluation by taking into account the total selenium content in the dialyzate as the sum of the different selenium species concentrations (mass balance study for selenium speciation) also proved good accuracy throughout the *in vitro* procedure for selenium species bio-availability (Supplementary Table S2).

4. Conclusions

Bioavailability of total selenium and selenium species ($SeCys_2$, SeMet and SeOMet), based on an *in vitro* approach consisting of using a dialysis membrane during the simulated intestinal digestion (dializability), was evaluated in different seafood products (mollusks, white fish and cold water fish). Low bioavailability percentages for total selenium (6.69 ± 3.39 and 5.45 ± 2.44% for fish and mollusk samples, respectively) were obtained. Similar bioavailability percentages was achieved for total selenium as a sum of selenium species ($SeCys_2$ + SeOMet and SeMet, mainly). HPLC–MS data confirmed SeMet oxidation during the *in vitro* procedure. Finally, accuracy of the *in vitro* procedure was assessed by means of a mass balance study. Good results have been obtained for total selenium bioavailability, and also for the bioavailability of the different selenium species present in the seafood samples studied.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.01.116.

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