JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Study of the Effect of Sample Preparation and Cooking on the Selenium Speciation of Selenized Potatoes by HPLC with ICP-MS and Electrospray Ionization MS/MS

HEIDI GOENAGA INFANTE,*,† ANA ARIAS BORREGO,‡ EMMA PEACHEY,† RUTH HEARN,† GAVIN O'CONNOR,† TAMARA GARCÍA BARRERA,‡ AND JOSÉ LUIS GÓMEZ ARIZA‡

LGC Limited, Queens Road, Teddington, Middlesex TW11 OLY, United Kingdom, and Departamento de Química y Ciencia de los Materiales, Facultad de Ciencias Experimentales, Universidad de Huelva, Campus de El Carmen, 21007 Huelva, Spain

The efficiency of enzymatic hydrolysis and leaching with water using accelerated solvent extraction (ASE) or boiling was investigated for quantitative Se speciation in selenized potatoes using reversed phase HPLC coupled to ICP-MS. Preliminary identification of selenomethionine (SeMet), Se-methylselenocysteine (SeMeCys), and selenate in extracts of potato skin and flesh was achieved using complementary reversed phase and anion-exchange HPLC-ICP-MS and retention time matching with standards. The quantitative speciation data revealed a higher percentage of selenomethionine (73% of the total Se) found in the flesh in comparison with skin (containing 21% of the total Se as SeMet). ASE and boiling in water were found to be similar in terms of Se extraction efficiency and profiles. However, ASE was found to be more efficient than boiling with respect to sample cleanup and reduced sample handling. The presence of SeMet at parts per billion levels in selenized potatoes was confirmed by reversed phase HPLC with online ESI MS/MS.

KEYWORDS: Selenized potatoes; accelerated solvent extraction; enzymatic hydrolysis; water extract; Se-methyl-selenocysteine; selenomethionine; potato skin; potato flesh; HPLC-ICP-MS; HPLC-ESI MS/MS; selected reaction monitoring

INTRODUCTION

Selenium is an essential nutrient for humans (*1*). However, the content of this mineral in the ordinary diet in Europe and other parts of the world is insufficient, a fact that has engaged pharmaceutical and food companies in the production of Se-enriched food and supplements in an effort to increase Se intake levels (*2*−*5*). A long-term solution to increase Se intake levels has been the enrichment of crops with Se-containing fertilizers (*6*−*8*). Regulation of the manufacture of specific Se species in food would enable better safety and quality-control measures to be implemented. There is also a need to know to which Se species beneficial or detrimental health effects can be attributed (*3*, *5*, *9*, *10*). Currently there is a lack of this relevant information.

The need for characterization of a new range of Se-enriched food in terms of measurement and identification of different chemical forms of Se has led to development of state-of-the-art analytical techniques; must successful strategies are based on the complementary use of elemental and molecular mass spectrometry with high-resolution separation techniques (*7*, *8*, *11*−*15*).

A key outstanding challenge is the clear tradeoff between Se extraction efficiency and preservation of identity of the compounds, which is associated with most of the existing extraction/digestion procedures used prior to quantification by HPLC-ICP-MS. Extraction methods that mimic the physiological conditions in the body and/or food cooking in real life are of paramount importance to obtain useful speciation information from food analysis (*3*, *5*, *9*, *10*). Without such knowledge, false conclusions may be drawn when optimal selenium nutrition is assessed.

Table 1. Concentrations of Total Se and Selenomethionine in Potato Water Extracts Obtained by ASE and Boiling in Water[a]

	total Se		SeMet (as Se)	
potato sample	boiling (ng/g)	ASE (ng/g)	boiling (ng/g)	ASE (ng/g)
skin	814 ± 17	815 ± 17	176 ± 8	183 ± 8
flesh	398 ± 12	402 ± 11	321 ± 14	321 ±15

[a] Precisions (as SD) are calculated from three independent determinations.

* Corresponding author (telephone +44 (0) 20 8943 7555; fax +44 (0) 20 8943 2767; e-mail Heidi.Goenaga-Infante@lgc.co.uk).
† LGC Limited.
‡ University of Huelva.

10.1021/jf802650q CCC: $40.75
Published on Web 12/18/2008

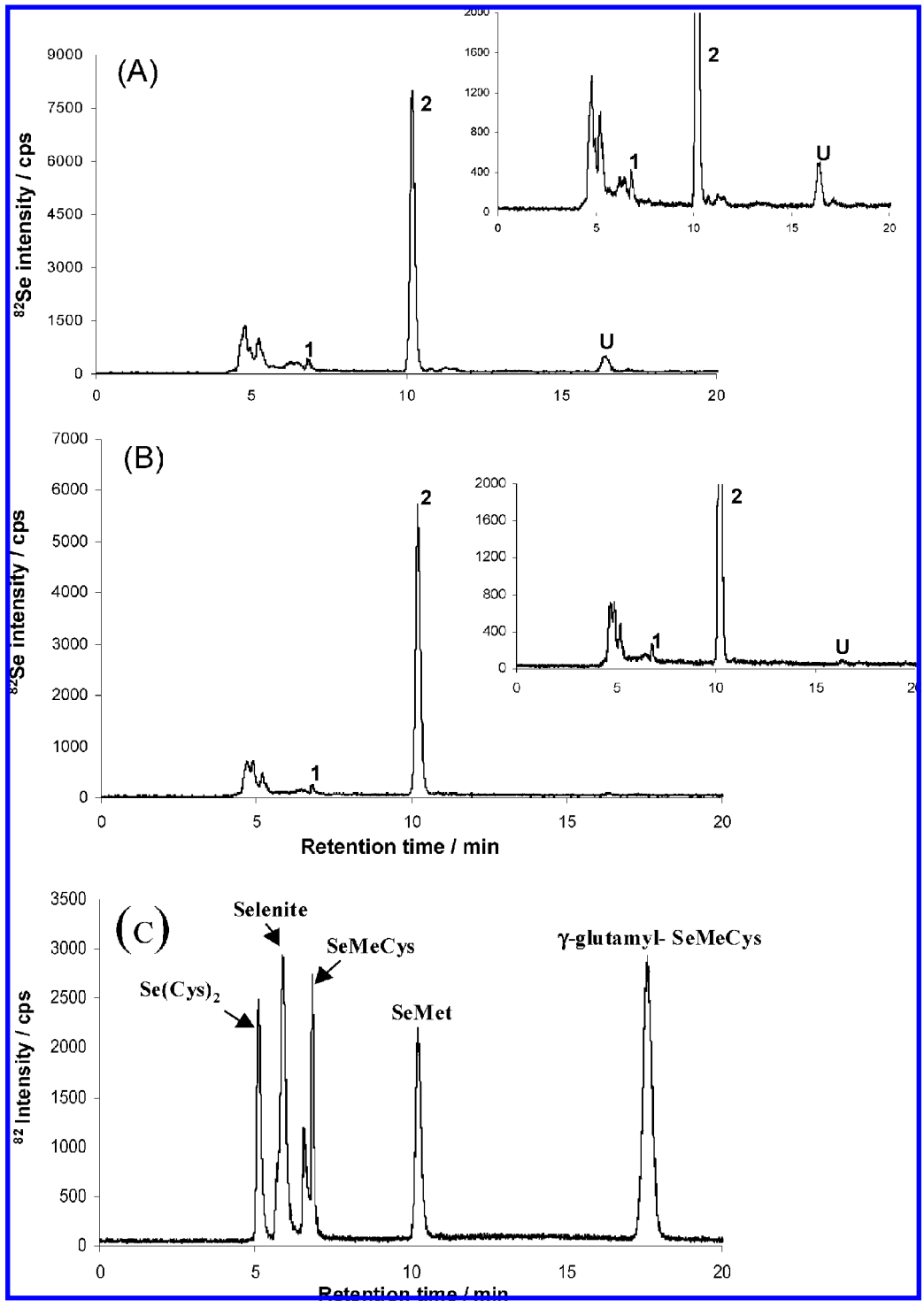

Figure 1. Elution profiles of Se by reversed phase HPLC-ICP-MS for the enzymatic extracts from (**A**) potato skin and (**B**) flesh and for (**C**) a Se standard mixture containing 3 μg L^{-1} Se as selenocystine (Se(Cys)$_2$), 2.5 μg/L Se as selenite, 5 μg/L Se as SeMeCys and SeMet, and 25 μg/L Se as γ-glutamyl-SeMC. (Insets) Chromatograms with an expanded scale. Peak identification in sample chromatograms: 1, SeMeCys; 2, SeMet; U, unknown Se peak.

Hydrolysis with proteolytic enzymes, which mimics the physiological conditions in the body, or acid hydrolysis with methanesulfonic acid, a harsher chemical extraction, has led to the highest extraction efficiencies of Se (mostly as SeMet) from Se-enriched food and supplements (*16*−*26*). Moreover, previous studies have suggested that cooking often reduces the Se content of most foods. For example, losses of Se of approximately 40 and 85% from mushrooms and garlic have been reported during boiling owing to leaching with water (*3*, *15*).

This study was designed to investigate for the first time the effect of different extraction and cooking procedures on the Se species distribution obtained by HPLC-ICP-MS and HPLC-ESI MS/MS in selenized potatoes for human consumption, which were grown in the presence of Se-containing fertilizers. The efficiency of extraction methods such as hydrolysis with proteolytic enzymes, which mimics the physiological conditions in the body, and leaching with water using accelerated solvent extraction (ASE) or boiling, which mimics food cooking in real life, for quantitative Se speciation in potato skin and flesh by complementary reversed phase and anion-exchange HPLC coupled with ICP-MS has been investigated. Structural confirmation of the major Se species detected by HPLC-ICP-MS in

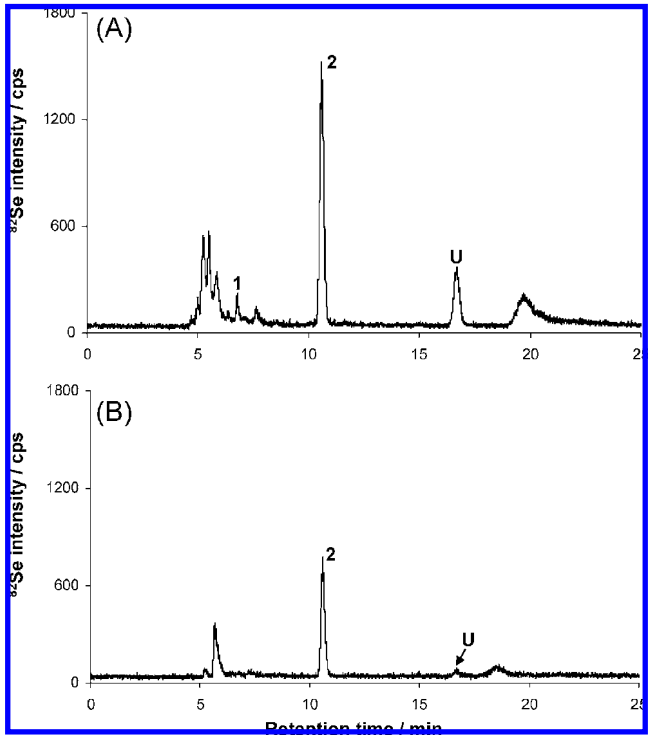

Figure 2. Elution profiles of Se by reversed phase HPLC-ICP-MS for the aqueous extracts from (**A**) potato skin and (**B**) flesh. Peak identification: 1, SeMeCys; 2, SeMet; U, unknown Se peak.

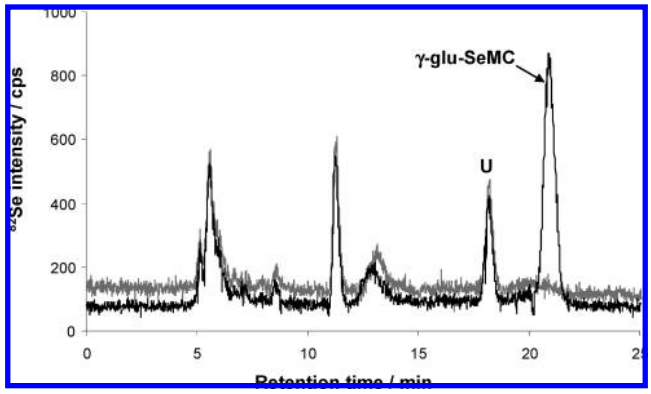

Figure 3. HPLC-ICP-MS chromatograms of a (1:3) diluted unspiked skin aqueous extract (gray) and of a (1:3) diluted skin aqueous extract spiked with a standard of γ-glutamyl-Se-methyl-selenocysteine (black).

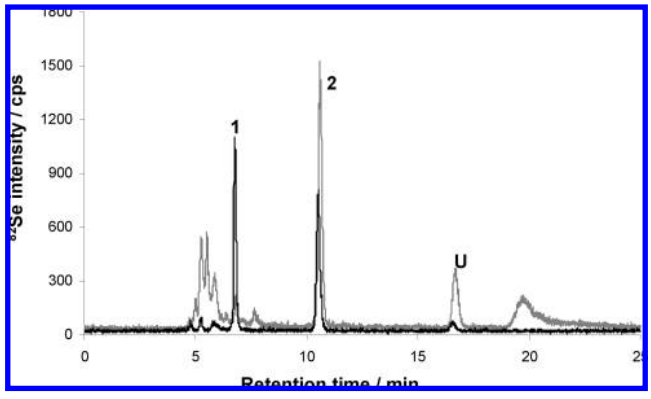

Figure 4. Se profiles obtained by HPLC-ICP-MS for the partially purified skin fraction (black) and for the whole skin extract (gray).

potato enzymatic extracts was addressed using reversed phase HPLC in online combination with ESI MS/MS in selected reaction monitoring mode. To do this, for species present at low concentrations (minor Se peaks), it was necessary to develop a sample cleanup/preconcentration method.

MATERIALS AND METHODS

Instrumentation. Extraction of the water-soluble seleno compounds from potato was carried out by accelerated solvent extraction (ASE) using a Dionex ASE 200 system (Sunnyvale, CA) or cooking in boiling water. Enzymatic hydrolysis of samples was performed using a hybridization oven model HB-2 (Techne, Duxford, Cambridge, U.K.), in which homogenization of samples and the buffered enzyme can be accomplished during incubation.

For acidic digestion of the solid sample a microwave oven model Multiwave 3000 (Anton Paar, Graz, Austria) was employed.

HPLC-ICP-MS measurements were performed using an Agilent Technologies 1200 HPLC system (Palo Alto, CA) for chromatographic separations and an Agilent 7500i ICP-MS for element-specific detection. Reversed phase HPLC was performed on a 250 mm × 4.6 mm i.d, 5 μm, Zorbax Rx-C_8 column (Agilent). Anion-exchange HPLC was performed on a 250 mm × 4.1 mm i.d., 10 μm, PRP-X 100 column (Hamilton Co., Reno, NV). The HPLC column was directly connected to the 100 μL/min PFA microflow concentric nebulizer of the ICP-MS via 30 cm × 0.1 mm i.d. PEEK tubing. Agilent Technologies ICP-MS chromatographic software (G1824C version C.01.00) was used for integration of the chromatographic signal. The optimum ICP-MS settings and HPLC conditions were as follows: ICP-MS RF power, 1200 W; plasma Ar flow rate, 15 L/min; makeup Ar flow rate, 0.29 L/min; nebulizer Ar flow rate, 0.89 L/min; spray chamber temperature, 2 °C; isotopes monitored, ^{77}Se, ^{82}Se; points per spectral peak, 1; integration time per mass, 100 ms; HPLC mobile phase, 0.1% formic acid, 2% (v/v) MeOH; flow rate, 0.5 mL/min; and injection loop, 50 μL.

For the HPLC-ESI MS/MS experiments, a 4000 QTRAP mass spectrometer (ABI/MDS Sciex) and an Agilent Technologies 1100 HPLC system were used. The effluent of the reversed phase HPLC column (0.5 mL/min) was fed directly into the electrospray source using 50 cm × 0.1 mm i.d. PEEK connecting tube. Confirmation of the presence of SeMet was on the basis of chromatographic retention time while the MS was operated in SRM mode. Data acquisition and processing were performed using ABI Analyst software version 1.4.1. The optimum ESI MS/MS instrumental parameters for online measurements with HPLC were as follows: mode, positive ion; source, turbo V ion spray; capillary voltage, 3.2 kV; desolvation temperature, 700 °C; nebulization gas, 80 psi; turbo gas, 50 psi; curtain gas, 35 psi; declustering potential, 30 V; collision energy, 24 eV; and collision gas, nitrogen.

Reagents, Standards, and Samples. Chemical substances were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. The standard of L-γ-glutamyl-Se-methylseleno-L-cysteine was purchased from PharmaSe (Lubbock, TX). A single-standard stock solution (1 mg/g) was prepared by dissolving the Se standard substance in ultrapure water. This stock solution was kept at 4 °C in the dark.

Methanol (Fisher Scientific, Loughborough, U.K.) was of HPLC grade. All of the reagents used were of the highest available purity. Deionized water (18 MΩ cm) was obtained from an Elga water purification unit (Elga, Marlow, Buckinghamshire, U.K.). Superpurity concentrated nitric acid was purchased from Romil (Cambridge, U.K.).

A standard solution of 10 μg/kg of Se in the corresponding mobile phase was prepared from a 1000 mg/kg Se reference solution (Romil) and used for the daily optimization of the ICP-MS parameters.

Selenized potatoes grown in the presence of Se-containing fertilizers were kindly provided by Mark Law from Nutrilaw Ltd. (Narborough, Norfolk, U.K.). Potatoes were peeled. and the flesh was cut into small pieces. Skin and flesh fractions were freeze-dried, ground, thoroughly homogenized, treated, and analyzed as independent samples. Sample homogenates were stored in the dark at −80 °C before extraction of Se compounds from the solid matrix was undertaken.

Procedures. *Determination of Total Se.* Half a gram of potato skin or flesh was accurately weighed into a Teflon microwave digestion vessel. Digestion was performed using a nitric acid/hydrogen peroxide

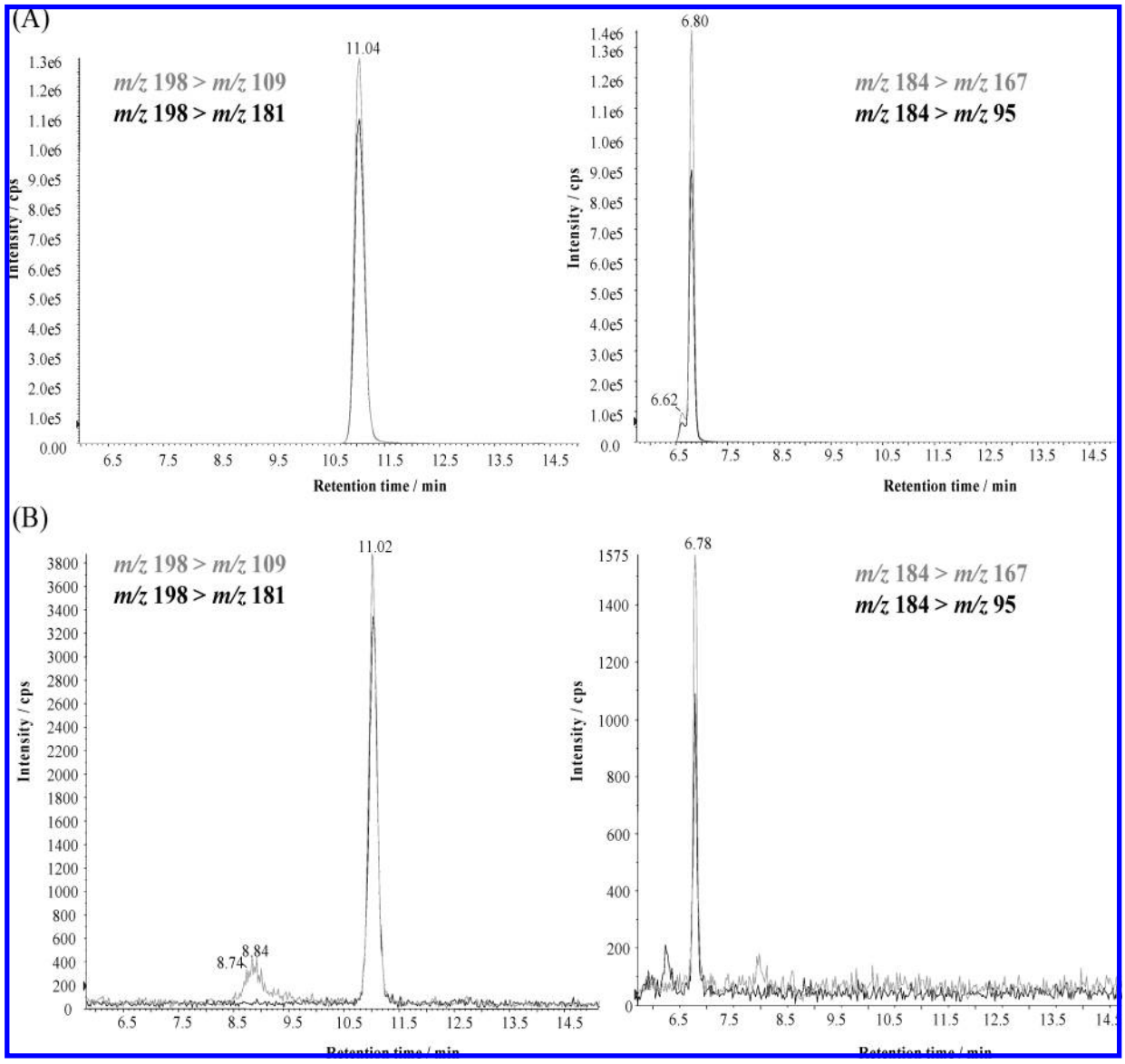

Figure 5. HPLC-ESI MS/MS chromatograms (SRMs of four transitions) of (**A**) a selenomethionine and Se-methyl-selenocysteine standard mixture and (**B**) the partially purified skin fraction.

(1:1, v/v) solution in a microwave oven, as described previously (*23*). Digested samples were appropriately diluted with ultrapure water before analysis by ICP-MS. Quantification was performed by external calibration, monitoring the isotopes ^{77}Se and ^{82}Se and using Ge and Rh as internal standards. The isotope ratio ^{77}Se/^{82}Se was also monitored to check for possible matrix-induced interferences (e.g., Cl- or Br-based interferences) on the detection of such Se isotopes. A cod muscle CRM 422 (IRMM, Geel, Belgium) with a certified Se concentration value of 1.63 ± 0.07 μg/g for Se was used for evaluation of the accuracy of the procedure.

Extraction of Water-Soluble Selenium Species. Two extraction procedures (ASE and boiling in water) were evaluated. For determination of total Se, the 1:10 diluted extract was analyzed by ICP-MS as described above. For Se speciation analysis, the extract was filtered (0.45 μm acetate cellulose filter) and diluted with ultrapure water just before it was applied to the HPLC column.

Accelerated Solvent Extraction. Flesh (0.2 g) and skin (0.3 g), accurately weighed, were submitted to extraction with water using an ASE system and the procedure described previously (*27*).

Boiling in Water. Flesh (0.2 g) or skin (0.3 g) was accurately weighed into a 100 mL Pyrex reaction vessel. The vessels were placed on a hotplate, and water was left to boil for 30 min. Extracts were cooled to room temperature, and a 10 mL aliquot was then carefully pipetted into a 10 mL tube to separate the aqueous extract to be analyzed for total Se and Se speciation from the floating solid residue because this could not be simply achieved by centrifugation.

Extraction of Protein-Bound Selenium. To release protein-bound selenium, enzymatic hydrolysis with protease, lipase, and Driselase was the method of choice. Forty milligrams of protease and 20 mg of lipase in 5 mL of a previously degassed 30 mmol/L Tris-HCl buffer solution (pH 7.5) were added to 0.4 g of skin or flesh. Incubation at 37 °C was then carried out in the dark for 20 h. During enzymolysis, the sample slurries were constantly and gently homogenized, using a rotary shaker set at 60 rpm. Hydrolyzed samples were centrifuged at 3000 rpm for 30 min and the supernatants filtered and stored at −20 °C. The residue was then subjected to proteolytic digestion with one more fresh buffered enzymatic solution also containing 100 mg of Driselase, used to release cell wall-bound components. Finally, the two supernatants were pooled, filtered, and appropriately diluted with water before Se speciation analysis.

Se Speciation Analysis by HPLC-ICP-MS. For determination of SeMet and SeMeCys, a 50 μL portion of the 1:2 diluted extract was analyzed by RP HPLC-ICP-MS at the flow rate of 0.5 mL/min using a water/methanol (98:2, v/v) mixture containing 0.1% (v/v) formic acid as the mobile phase. Anion-exchange HPLC-ICP-MS was used for determination of selenate in enzymatic extracts of skin and flesh. To do this, 50 μL of the digest was injected, and elution was achieved by using 5 mM ammonium hydrogencitrate (pH 5.9) in 2% (v/v) methanol

at 0.9 mL/min. Calibration was carried out by the standard addition technique at three concentration levels, using peak area measurements of the chromatographic signals by monitoring the ^{82}Se signal. The standard solution used for calibration was characterized for its total Se content by ICP-MS.

RP HPLC-ESI MS/MS Analysis. The effluent of the reversed phase HPLC column was fed directly into the electrospray source. Confirmation of the presence of SeMet and SeMeCys was on the basis of chromatographic retention time while the MS was operated in selected reaction monitoring (SRM) mode. The most abundant transitions m/z 198 > m/z 181 (loss of OH) and m/z 198 > m/z 109 (formation of the ion fragment $CH_3SeCH_2^+$) and m/z 184 > m/z 167 (loss of OH) and m/z 184 > m/z 95 (formation of the ion fragment $SeCH_3^+$) were monitored for SeMet and SeMeCys, respectively.

Collection and Lyophilization of the Unknown Se Fraction. Freshly 1:2 diluted skin digests of 50 μL were applied to the RP-HPLC column. HPLC separation was carried out as described above. For partial purification of the unknown Se fraction (retention time of 16.7 min) the HPLC effluent was collected in vials during 0.8 min (between 16.4 and 17.2 min) for every chromatographic run replicate. The collected fractions from 17 independent injections were pooled and freeze-dried. Dissolution of the lyophilisate in 100 μL of water was performed prior to its analysis by RP HPLC-ESI MS/MS.

RESULTS AND DISCUSSION

Total Se Content of Selenized Potatoes. As a first step, the potato flesh and skin were analyzed for their total Se content using the procedure described above. The concentration of Se found in the skin (1990 ± 40 ng/g Se) was approximately 3-fold higher than that found in the flesh (670 ± 20 ng/g Se). Precisions (as SD) were calculated from six independent determinations ($n = 6$). Recovery of total Se from the cod muscle CRM averaged 98 ± 3% ($n = 4$).

HPLC-ICP-MS Analysis of Enzymatic Hydrolysates. Extraction with proteolytic enzymes, which mimics the physiological conditions in the human intestine, has been the most widely used approach to release protein-bound SeMet from Se-enriched food and supplements (*17, 22, 25, 28*).

HPLC-ICP-MS chromatograms of enzymatic extracts of potato skin and flesh obtained by enzymatic hydrolysis under the conditions given above are shown in **Figure 1**. The HPLC chromatogram obtained for a selenium standard mixture is also given in this figure. On the basis of retention time matching with Se standards, SeMet (peak 2) could be identified as the major species in enzymatic hydrolysates of both skin and flesh. Also, both extracts seemed to contain SeMeCys (peak 1) as a minor species. The chromatograms in **Figure 1** also show an unknown Se peak (U) at 16.7 min (with the highest abundance in skin), which could not be identified by spiking experiments.

The concentrations of selenomethionine and selenate found in potato skin and flesh by HPLC-ICP-MS following multistep enzymatic extraction were 420 ± 20 ng/g (SeMet, as Se) and 154 ± 16 ng/g (selenate) and 490 ± 30 ng/g (SeMet, as Se) and 44 ± 4 ng/g (selenate), respectively. Precisions (as SD) are calculated from three independent determinations. Se relative detection limits (3σ criterion for three replicates in each case) obtained for SeMeCys and SeMet (by reverse phase HPLC-ICP-MS) and for selenate (by anion-exchange HPLC-ICP-MS) were 10.1 ± 0.5, 14.5 ± 0.7, and 6.3 ± 0.3 ng/kg, respectively. Recoveries of the spiked SeMet and selenate averaged 95 ± 5 and 101 ± 8%, respectively. Whereas SeMet appears to be the major Se species in flesh (73% of the total Se), it seems to comprise only 21% of the total Se in skin. The potato flesh and skin were also found to contain 6 and 8%, respectively, of the total Se as selenate. Clearly, a high percentage of Se (approximately 70% of the total Se) associated with other Se species (including peak U) in the skin remains undetected and/or unidentified using this methodology.

Comparison of ASE and Boiling in Water for HPLC-ICP-MS Analysis of Water-Soluble Selenium. Boiling potatoes in water is a common cooking procedure in real life. On the other hand, ASE is an automated, high-throughput operation technique used for the extraction of solid and semisolid sample matrices using common solvents at elevated temperatures and pressures with reduced sample handling. Moreover, ASE (at temperatures between 100 and 115 °C) was found to offer clear advantages in terms of automation and sample throughput in comparison with conventional methods (e.g., the standard sonication method) for extraction of Se compounds from yeast materials in water. This was achieved without sacrifice in selenium recovery and speciation integrity (*18*).

In this study, ASE (at 100 °C) is compared in terms of extraction efficiency of water-soluble total Se and Se species with potato cooking by boiling in water. As a first step, the potato extracts obtained using these procedures at extraction/cooking times of 15, 30, and 45 min were analyzed for their total Se content. Recovery of total Se from flesh and skin was found to increase from 50.9 ± 2.5 and 31.0 ± 1.7%, respectively (for 15 min extraction/cooking time), to 59.7 ± 2.8 and 40.9 ± 2.0%, respectively, for a total extraction/cooking time of 30 min (two ASE cycles, each of 15 min). Because longer times of extraction/cooking did not further improve recovery of total Se from dry potato samples, an extraction/cooking time of 30 min was selected as optimal for further work.

Table 1 summarizes the results obtained for total Se and SeMet in potato extracts obtained by ASE and boiling in water under optimal conditions. As can be seen, the concentrations of both Se and SeMet using the two extraction methods agree very well. As noted earlier, losses of about 60 and 41% of the total Se from flesh and skin, respectively, owing to leaching in water were observed. It is interesting to note that approximately 80% of the total water-soluble Se from flesh can be found as SeMet. ASE was, however, found to be more efficient than boiling with respect to sample cleanup and reduced sample handling because this system integrates sample extraction and cleanup, which are performed within the extraction cell, producing a clean extract that is ready for speciation analysis. Therefore, ASE was selected for further experiments.

The HPLC-ICP-MS chromatograms obtained for water extracts from potato skin and flesh are shown in **Figure 2**. As shown, Se seems to be associated with different chromatographic fractions; two of these compounds can be tentatively identified as SeMeCys (peak 1 at 6.8 min) and SeMet (peak 2 at 11 min) on the basis of comparison with the corresponding retention times for known standards. As previously observed for the enzymatic extracts, an unknown Se peak at 16.7 min (with the highest abundance in skin) could be detected by HPLC-ICP-MS. As previously reported (*27*), the standard of γ-glutamyl-Se-methyl-selenocysteine appears to elute from the reversed phase column at a retention time very similar to that of the investigated unknown Se peak under the conditions described. Moreover, like the unknown peak at $t_R = 16.7$ min, the dipeptide species is known to occur in Se food/supplements not incorporated into proteins but easily extractable by leaching into water (*27*). For these reasons, investigation on the origin of this water-soluble Se species ($t_R = 16.7$ min) was attempted by HPLC-ICP-MS after spiking of the skin extract with a standard of γ-glutamyl-Se-methyl-selenocysteine. The results are shown in **Figure 3**, which reveal that the unknown Se peak does not

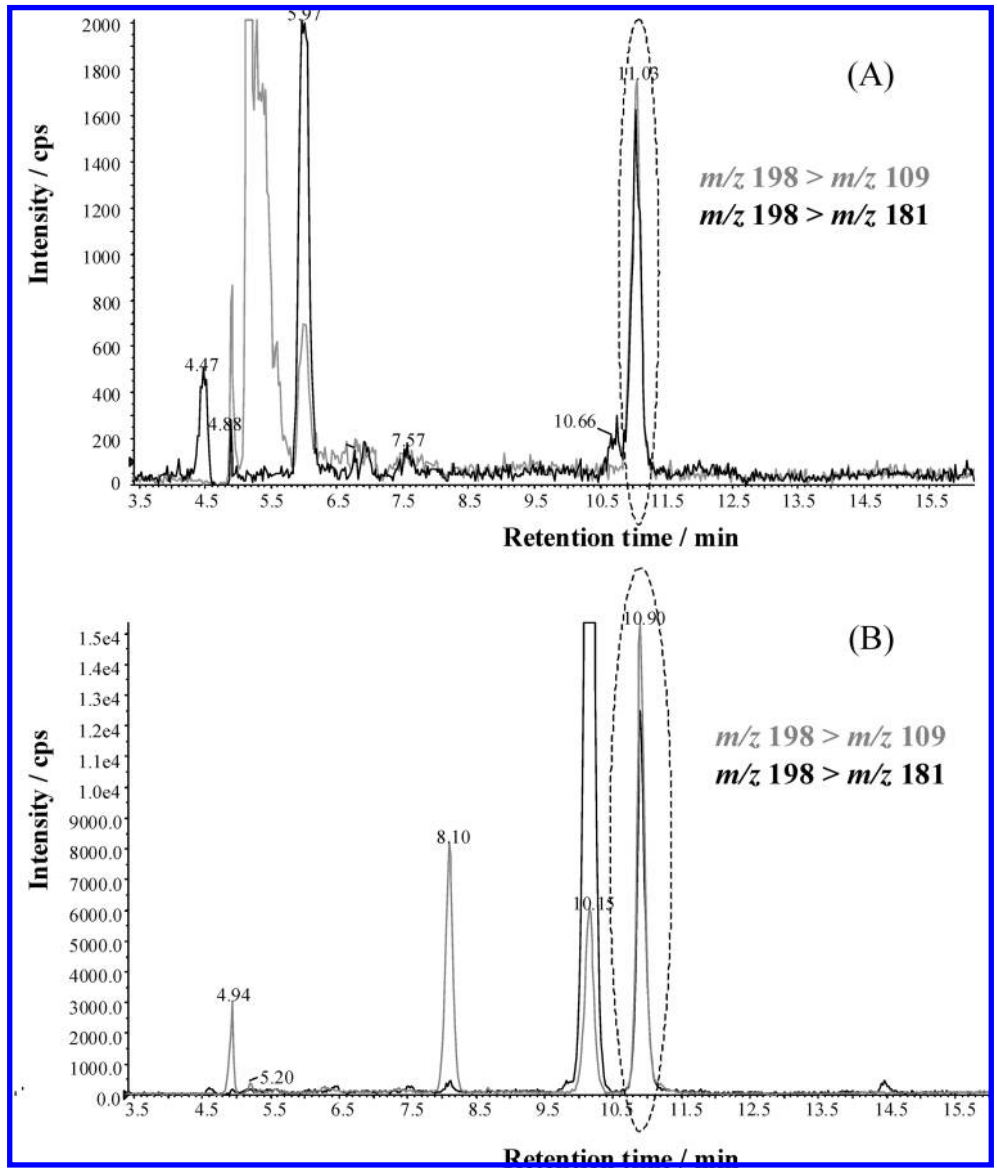

Figure 6. HPLC-ESI MS/MS chromatograms (SRMs of two transitions) of (**A**) an aqueous extract and (**B**) an enzymatic extract of selenised potatoes.

correspond to γ-glutamyl-Se-methyl-selenocysteine (t_R = 17.8 min). To obtain information on the identity of this unknown minor Se species (t_R = 16.7 min), fraction collection and lyophilization were performed prior to its analysis by online HPLC with parallel ICP-MS and ESI MS/MS.

As a first step, the speciation analysis of Se in the partially purified fraction of potato skin was undertaken using RP HPLC combined with ICP-MS. The speciation results shown in **Figure 4** point to the presence of two main Se peaks; assignments based on retention times suggest that these Se species correspond to SeMeCys (peak 1) and SeMet (peak 2). The chromatogram of the whole skin extract is also shown for comparison with the elution profile of Se for the partially purified fraction. Results also show that the peak area of the Se species with a retention time of 16.7 min (unknown Se compound) decreased for the collected/preconcentrated fraction, suggesting transformation or breakdown of the unknown Se species, probably occurring during sample cleanup and/or preconcentration. To further verify this, the Zorbax Rx-C_8 HPLC column was connected to the ESI MS/MS instrument and the analysis of the partially purified fraction of potato skin was carried out in SRM mode.

Se Compound Identification by HPLC-ESI MS/MS. *Investigation of the Origin of the Unknown Se Peak (U).* Specific transitions from the precursor ion to product ions (obtained by CID) were selected to be monitored by MS/MS on the basis of the product ions found for the SeMet and SeMeCys peak in standard solutions (*18*). **Figure 5** shows the SRMs of the most abundant transitions *m*/*z* 184 > *m*/*z* 167 and *m*/*z* 184 > *m*/*z* 95 and *m*/*z* 198 > *m*/*z* 181 and *m*/*z* 198 > *m*/*z* 109 obtained for standard solutions of SeMeCys and SeMet, respectively (**Figure 5A**) and for the collected skin fraction (**Figure 5B**), which occur at the retention time of SeMeCys (6.8 min) and SeMet (11.0 min). The relative abundance ratio of the two transitions obtained for the skin fraction was found to match the comparison standards within ±4%. This is consistent with the presence of SeMet and SeMeCys in the investigated skin fraction, as detected by HPLC-ICP-MS. Further studies involving development of more efficient sample cleanup/preconcentration strategies in combination with HPLC-ESI MS/MS should be pursued to elucidate the structure of the unknown Se peak (16.7 min) detected in potato skin and, to a lesser extent, in flesh extracts by HPLC-ICP-MS.

Confirmation of the Presence of SeMet as a Major Compound in Selenized Potatoes. To verify the presence of SeMet in selenized potatoes, the water and enzymatic extracts were further analyzed by online RP HPLC-ESI MS/MS. **Figure 6** shows the

SRMs of the most abundant transitions obtained for an aqueous extract (**Figure 6A**) and an enzymatic extract (**Figure 6B**) from selenized potatoes, which occur at the retention time of those obtained for a SeMet standard (see **Figure 5A**). It is also clear that another peak (retention time of 10 min) for the two transitions appears in the chromatogram of the enzymatic extract. This peak corresponds to ions having the same *m*/*z* and losing a fragment of the same *m*/*z*, but the relative abundance ratio of the two transitions at $t_R = 10$ min did not agree with that of the SeMet standard. However, for both extracts, the relative abundance ratio of the two transitions at $t_R = 11$ min was found to match the comparison standard within ±5%. This is considered to be the first proof of identity of SeMet in selenized potatoes.

The complementary use of molecular and element-specific mass spectrometry with liquid chromatography offers a promising and straightforward approach that can be applied in the future to the investigation of Se species distribution in other complex food-type samples. It is clear that the use of a selective chromatographic method compatible with both ICP and ESI, which has advantages in terms of robustness, simplicity of sample preparation, and online detection/identification capabilities over multistep separation/purification approaches, is of paramount importance to obtain useful speciation information from food analysis.

ACKNOWLEDGMENT

We are grateful to Mark Law of Nutrilaw Ltd., U.K., for providing the selenized potatoes used in this study.

LITERATURE CITED

(1) Rayman, M. P. The importance of selenium to human health. *Lancet* **2000**, *356*, 233–241.

(2) Kirby, J. K.; Lyons, G. H.; Karkkainen. Selenium speciation and bioavailability in biofortified products using species-unspecific isotope dilution and reverse phase ion pairing-inductively coupled plasma-mass spectrometry. *J. Agric. Food Chem.* **2008**, *56*, 1772–1779.

(3) Rayman, M. P.; Goenaga-Infante, H.; Sargent, M. Food-chain selenium and human health: spotlight on speciation. *Br. J. Nutr.* **2008**, *100*, 238–253.

(4) Hinojosa Reyes, L.; Marchante-Gayón, J. M.; García Alonso, J. I.; Sanz-Medel, A. Application of isotope dilution analysis for the evaluation of extraction conditions in the determination of total selenium and selenomethionine in yeast-based nutritional supplements. *J. Agric. Food Chem.* **2006**, *54*, 1557–1563.

(5) Goenaga-Infante, H.; Hearn, R.; Catterick, T. Current mass spectrometry strategies for selenium speciation in dietary sources of high-selenium. *Anal. Bioanal. Chem.* **2005**, *382*, 957–967.

(6) Lyons, G.; Stangoulis, J.; Graham, R. High-selenium wheat: biofortification for better health. *Nutr. Res. Rev.* **2003**, *16*, 45–60.

(7) Larsen, E. H.; Lobinski, R.; Burger-Meÿer, K.; Hansen, M.; Ruzik, R.; Mazurowska, L.; Rasmussen, P. H.; Sloth, J. J.; Scholten, O.; Kik, C. Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate. *Anal. Bioanal. Chem.* **2006**, *385*, 1098–1108.

(8) Warburton, E.; Goenaga-Infante, H. Methane mixed plasma-improved sensitivity of inductively coupled plasma mass spectrometry detection for selenium speciation analysis of wheat-based food. *J. Anal. Atom. Spectrom.* **2007**, *22*, 370–376.

(9) Dumont, E.; Vanhaecke, F.; Cornelis, R. Selenium speciation from food source to metabolites: a critical review. *Anal. Bioanal. Chem.* **2006**, *385*, 1304–1323.

(10) Gammelgaard, B.; Gabel-Jensen, C.; Stürup, S.; Hansen, H. R. Complementary use of molecular and element-specific mass spectrometry for identification of selenium compounds related to human selenium metabolism. *Anal. Bioanal. Chem.* **2008**, *390*, 1691–1706.

(11) McSheehy, S.; Yang, W.; Pannier, F.; Szpunar, J.; Lobinski, R.; Auger, J.; Potin-Gautier, M. Speciation analysis of selenium in garlic by two-dimensional high performance liquid chromatography with parallel inductively coupled plasma mass spectrometric and electrospray tandem mass spectrometric detection. *Anal. Chim. Acta* **2000**, *421*, 147–153.

(12) Shah, M.; Kannamkumarath, S. S.; Wuilloud, J. C. A.; Wuilloud, R. G.; Caruso, J. A. Identification and characterization of selenium species in enriched green onion (*Allium fistulosum*) by HPLC-ICP-MS and ES-ITMS. *J. Anal. Atom. Spectrom* **2004**, *19*, 381–386.

(13) Dumont, E.; Ogra, Y.; Vanhaecke, F.; Suzuki, K. T. Liquid chromatography−mass spectrometry (LC-MS): a powerful combination for selenium speciation in garlic (*Allium sativum*). *Anal. Bioanal. Chem.* **2006**, *384*, 1196–1206.

(14) Dernovics, M.; Giusti, P.; Lobinski, R. ICP-MS-assisted nano-HPLC-electrospray Q/time-of-flight MS/MS selenopeptide mapping in Brazil nuts. *J. Anal. Atom. Spectrom.* **2007**, *22*, 41–50.

(15) Ogra, Y.; Ishiwata, K.; Ruiz Encinar, J.; Lobinski, R.; Suzuki, K. T. Speciation of selenium in selenium-enriched shiitake mushroom, *Lentinula edodes*. *Anal. Bioanal. Chem.* **2004**, *379*, 861–866.

(16) Ayouni, L.; Barbier, F.; Imbert, J. L.; Gauvrit, J.; Lantéri, P.; Grenier-Loustalot, M. F. New separation method for organic and inorganic selenium compounds based on anion exchange chromatography followed by inductively coupled plasma mass spectrometry. *Anal. Bioanal. Chem.* **2006**, *385*, 1504–1512.

(17) Larsen, E. H.; Hansen, M.; Paulin, H.; Moesgaard, S.; Reid, M.; Rayman, M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. *J. AOAC Int.* **2004**, *87*, 225–232.

(18) Goenaga Infante, H.; O'Connor, G.; Rayman, M.; Wahlen, R.; Entwisle, J.; Norris, P.; Hearn, R.; Catterick, T. Selenium speciation analysis of Se-enriched supplements by HPLC with ultrasonic nebulisation ICP-MS and electrostray MS/MS detection. *J. Anal. Atom. Spectrom.* **2004**, *19*, 1529–1538.

(19) Uden, P. C.; Boakye, H. T.; Kahakachchi, C.; Hafezi, R.; Nolibos, P.; Block, E.; Johnson, S.; Tyson, J. F. Element selective characterisation of stability and reactivity of selenium species in selenised yeast. *J. Anal. Atom. Spectrom.* **2004**, *19*, 65–73.

(20) Wrobel, K.; Kannamkumarath, S. S.; Wrobel, K.; Caruso, J. A. Hydrolysis of proteins with methanesulfonic acid for improved HPLC-ICP-MS determination of seleno-methionine in yeast and nuts. *Anal. Bioanal. Chem.* **2003**, *375*, 133–138.

(21) Yang, L.; Mester, Z.; Sturgeon, R. Determination of methionine and selenomethionine in yeast by species-specific isotope dilution GC/MS. *Anal. Chem.* **2004**, *76*, 5149–5156.

(22) Mester, Z.; Willie, S.; Yang, L.; Sturgeon, R.; Caruso, J. A.; Fernández, M. L.; Fodor, P.; Goldschmidt, R. J.; Goenaga-Infante, H.; Lobinski, R.; Maxwell, P.; McSheehy, S.; Polatajko, A.; Sadi, B. B.; Sanz-Medel, A.; Scriver, C.; Szpunar, J.; Wahlen, R.; Wolf, W. Certification of a new selenised yeast reference material (SELM-1) for methionine, selenomethionine and total selenium content and its use in an intercomparison exercise for quantifying these analytes. *Anal. Bioanal. Chem.* **2006**, *385*, 168–180.

(23) Díaz Huerta, V.; Hinojosa Reyes, L.; Marchante-Gayón, J. M.; Fernández Sánchez, M. L.; Sanz-Medel, A. Total determination and quantitative speciation analysis of selenium in yeast and wheat flour by isotope dilution analysis ICP-MS. *J. Anal. Atom. Spectrom.* **2003**, *18*, 1243–1247.

(24) Potalajko, A.; Sliwka-Kaszynska, M.; Dernovics, M.; Ruzik, R.; Encinar, J. R.; Szpunar, J. A systematic approach to selenium speciation in selenised yeast. *J. Anal. Atom. Spectrom.* **2004**, *19*, 114–120.

(25) Goenaga Infante, H.; Sturgeon, R.; Turner, J.; Hearn, R.; Sargent, M.; Maxwell, P.; Yang, L.; Barzev, A.; Pedrero, Z.; Cámara, C.; Díaz Huerta, V.; Fernández Sánchez, M. L.; Sanz-Medel, A.; Emese, K.; Fodor, P.; Wolf, W.; Goldschmidt, R.; Vacchina, V.; Szpunar, J.; Valiente, L.; Huertas, R.; Labarraque, G.; Davis, C.;

Zeisler, R.; Turk, G.; Rizzio, E.; Mackay, L. G.; Myors, R. B.; Saxby, D. L.; Askew, S.; Chao, W.; Jun, W. Total selenium and selenomethionine in pharmaceutical tablets: assessment of the state of the art of measurement capabilities through international intercomparison CCQM-P86. *Anal. Bioanal. Chem.* **2008**, *390*, 629–642.

(26) Bodo, E. T.; Stefanka, Z.; Ipolyi, I.; Soros, C.; Dernovics, M.; Fodor, P. Preparation, homogeneity and stability studies of a candidate LRM for Se speciation. *Anal. Bioanal. Chem.* **2003**, *377*, 32–38.

(27) Goenaga Infante, H.; O'Connor, G.; Rayman, M.; Spallholz, J. E.; Wahlen, R.; Hearn, R.; Catterick, T. Identification of water-soluble γ-glutamyl-Se-methylselenocysteine in yeast-based selenium supplements by reversed-phase HPLC with ICP-MS and electrospray tandem MS detection. *J. Anal. Atom. Spectrom.* **2005**, *20*, 864–870.

(28) Amoako, P. O.; Kahakachchi, C. L.; Dodova, E. N.; Uden, P. C.; Tyson, J. F. Speciation, quantification and stability of selenomethionine, *S*-(methylseleno)cysteine and selenomethionine Se-oxide in yeast-based nutritional supplements. *J. Anal. Atom. Spectrom.* **2007**, *22*, 938–946.

Received for review August 28, 2008. Revised manuscript received November 17, 2008. Accepted November 18, 2008. The work described in this paper was supported by the U.K. Department for Innovation, Universities & Skills as part of the National Measurement System Chemical & Biological Metrology Knowledge Base Programme.

JF802650Q