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Speciation of selenium in diet supplements by HPLC–MS/MS methods

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Abstract

HPLC–MS/MS methods are developed to study the speciation of selenium in food supplements. The species most commonly present in supplements are considered: seleno-L-methionine ($C_5H_{11}O_2NSe$), L-selenocystine ($C_6H_{12}O_4N_2Se_2$), phenyl-L-selenocysteine ($C_9H_{11}O_2$-NSe), methyl-seleno-L-cysteine ($C_4H_9O_2NSe$), methaneseleninic acid (CH_3SeO_2H), selenate (SeO_4^{2-}), selenocyanate ($SeCN^-$) and selenite (SeO_3^{2-}). The chromatographic analysis is performed on a Luna C18 stationary phase: for the separation of the organic species the mobile phase is a mixture of methanol and water both 0.05% in trifluoroacetic acid, while for the separation of inorganic species the mobile phase is a mixture of methanol and tetrabutylammonium hydroxide 1.0 mM aqueous solution. Gradient elution conditions are used in both the separations. Two mass spectrometry analyzers are employed for detection, namely an ion trap for determination of the aminoacidic species and a triple quadrupole for the inorganic ones.

The methods are applied in the analysis of six commercial food supplements bought in pharmacies and supermarkets and containing selenium under different speciation forms. Speciation results are compared with the data labelled and with the amount of total selenium, determined by ICP spectroscopy. It is worthwhile to underline that HPLC–MS analysis of supplements containing selenate and selenite, gives values respectively lower than those expected and non detectable values of selenite. On the basis of some experiments, these results are explained through the taking place of side reactions in which selenite and selenate are reduced by ascorbic acid that is present in the supplements at very higher amounts.

Keywords: Selenium speciation; HPLC–MS analysis; Diet supplements; Selenite; Selenate; Selenomethionine

1. Introduction

The nutritional importance of selenium is object of recent discussion. On one hand, selenium is essential to life, since it plays a protective role against the effects of heavy metals and its deficiency causes cardiomyopathy and osteoarthropathy (McLaughlin, Parker, & Clarke, 1999). On the other hand, selenium excess can induce selenium silicosis and cancer (Rayman, 2000). The range of concentration between deficiency and toxicity is very narrow and strongly depends on the chemical form under which the metal is present (Michalke, 1995). Also the bio availability ("the micro nourishing amount that is available to being assimilated in a physiologically useful form") (Van Campen & Glahn, 1999) depends on the speciation, so that it is not easy to individuate the correct dose. The Recommended Dietary Allowances (RDA) refers to total selenium. According to Dietary Reference Intake (DRI, 2000), RDA is 55 μg/day for adults; according to the German and Austrian Nutrition Society and Swiss Nutrition Association, it ranges between 30 and 70 μg/day, while in Australia varies from 10–15 μg/day for infants to 70–85 μg/day

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for adults (Smrkolj, Pograjc, Hlastan-Ribic, & Stibilj, 2005). The tolerable upper intake level for adults is set at 400 μg/day (Tinggi, 2003).

In the world, deficiency of selenium in diet is more common than its abundance and is often compensated through selenium-enriched food supplements. The easy access to supplements, also available in drugstores and supermarkets, makes their use uncontrolled and potentially dangerous. Many commercial formulations label only the total selenium content and not the speciation forms. But, while selenium aminoacids are easily metabolised and selenide behaves as antioxidant agent and is the precursor *in vivo* of selenium aminoacids, the effects of selenite and selenate are still in discussion. According to some authors, they can be dangerous (Jia, Li, & Chen, 2005; McLaughlin et al., 1999; Rossman & Uddin, 2004), while other authors report the high potentiality of selenite against some kinds of cancer (Lipinski, 2005).

Speciation methods present in literature are mainly based on HPLC technique interfaced with ICP and mass spectrometry detection (Auger, Yang, Arnault, Pannier, & Potin-Gautier, 2004; Tsopelas, Ochsenkuhn-Petropulou, Mergias, & Tsakanika, 2005; Wrobel et al., 2004), anion exchange RP–HPLC–ICP–MS (Cai, Cabanas, Fernandez-Turiel, Abalos, & Bayona, 1995; Chassaigne, Chery, Bordin, & Rodriguez, 2002; Ochseunkuhn-Petropoulou, Michalke, Kavouras, & Schramel, 2003), ion-pair RP–HPLC–ICP–MS (Zheng, Ohata, Furuta, & Kosmus, 2000), gel permeation HPLC with ultrasonic nebulizer high-power nitrogen microwave-induced plasma mass spectrometry (Chatterje, Shibata, Tao, Tanaka, & Morita, 2004), size-exclusion-anion exchange-ICP–MS (Chassaigne et al., 2002), size-exclusion and ion-exchange chromatography with ICP–MS detection (Moreno, Quijano, Gutierrez, Perez-Conde, & Camara, 2004), GC–MS methods after derivatisation (Hunter & Kuykendall, 2004; Iscioglu & Henden, 2004).

Our purpose is the development of an HPLC method hyphenated with mass spectrometry detection to evaluate the speciation of selenium in dietary supplements. The selenium species most commonly present in supplements are considered and namely: seleno-L-methionine ($C_5H_{11}O_2NSe$), L-selenocystine ($C_6H_{12}O_4N_2Se_2$), phenyl-L-selenocysteine ($C_9H_{11}O_2NSe$), methyl-seleno-L-cysteine ($C_4H_9O_2NSe$), methanseleninic acid (CH_3SeO_2H), selenate (SeO_4^{2-}), selenocyanate ($SeCN^-$) and selenite (SeO_3^{2-}). Chemical structures are reported in Tables 1a and 1b.

2. Materials and methods

2.1. Instrumentation

The analyses for the organic species were carried out by a Finnigan Mat Spectra System instrument (San Jose, CA, USA) equipped with a SCM1000 degasser, a P4000 gradient pump, an AS3000 autosampler, interfaced by the SN4000 module to a UV6000LP diode array detector and to an ESI–MS ion trap LCQ Duo detector.

The analyses for the inorganic species were carried out by a Varian HPLC–MS (Palo Alto, CA, USA) equipped with a 230 ternary pump, a 410-autosampler and a 1200 L triple quadrupole mass spectrometric detector and an ESI interface.

The ICP spectroscopic measurements were performed by an ICP OES Perkin Elmer Optima 2000 DV instrumentation (Norwalk, CT, USA), settled at 196.026 nm. Argon gas at 15.0 L/min was used for plasma, at 0.2 L/min as auxiliary gas and at 0.8 L/min as nebulizer. The torch power was 1300 W. The flow-rate of the peristaltic pump was 1.5 mL/min. The sample was introduced by Venturi cyclonic nebulizer.

A microprocessor pH meter (Hanna Instrument, Portugal), equipped with a combined glass–calomel electrode, was employed for pH measurements.

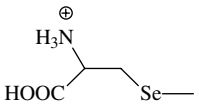
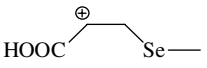
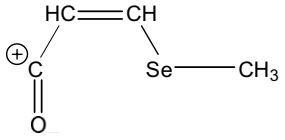
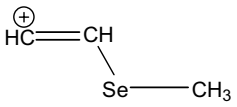
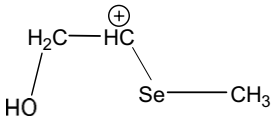
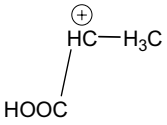
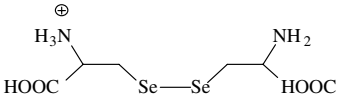
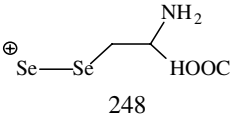
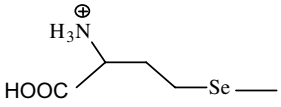
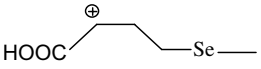
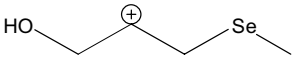
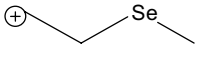
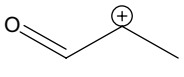
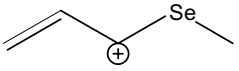
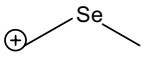
A Dionex ion chromatograph equipped with a GP40 gradient pump, ED40 electrochemical detector, LC20 chromatography enclosure and Consta Metric 3200 Thermo separator was employed in the ion-chromatographic analysis of selenite and selenate.

2.2. Reagents

Ultra pure water from a Millipore Milli-Q system (Milford, MA, USA) was used for the preparation of all solutions. HPLC-grade methanol from Merck (Darmstadt, Germany) was filtered through 0.45 μm membrane (Millex, Millipore). Tetrabutylammonium (TBA) hydroxide, octylamine (⩾98%), *o*-phosphoric acid (85%) were from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) 99% from Riedel de Haen (Seelze, Germany). Phenyl-L-selenocysteine (⩾95%) and methyl-seleno-L-cysteine (⩾98%), Na_2CO_3(⩾99%) and $NaHCO_3$ (⩾99%) were purchased from Fluka (Buchs, Switzerland). L-Selenocystine (⩾98%), seleno-L-methionine (98%), methaneseleninic acid (95%), sodium selenite (99%), potassium selenocyanate (97%) and sodium selenate (98%) were from Sigma–Aldrich (Milwaukee, WI, USA). 37% HCl, 65% HNO_3 were purchased from Merck (Darmstadt, Germany), argon and helium from SIAD (Bergamo, Italy). H_2SO_4 (96%) was acquired from Carlo Erba (Milan, Italy).

Standard solutions (50.0 mg/L) of phenyl-L-selenocysteine, methyl-seleno-L-cysteine and seleno-L-methionine were prepared in methanol. The standard solution of L-selenocystine was prepared (50.0 mg/L) in water added with trifluoroacetic acid to pH around 3.0. All the solutions were preserved at 4 °C in dark glass bottles, except seleno-L-methionine that was stored in freezer at −20 °C. The standard solutions of methaneseleninic acid, sodium selenite and potassium selenocyanate were prepared (1000.0 mg/L) in water and preserved at 4 °C in dark glass bottles; all the dilutions were performed in the mobile phase at its initial composition.

Table 1a
MS^n fragmentation pattern of the organic selenium species

Analyte (molecular mass)	Precursor ion	MS^2 product ion	MS^3 product ion	MS^4 product ion
Methyl-seleno-L-cysteine (182.07 amu)	 184	 167	 149	 121
			 139	
			 73	
L-Selenocystine (334.09 amu)	 337	 248		
Seleno-L-methionine (196.11 amu)	 198	 181	 153	 123
				 57
			 135	
			 109	

(*continued on next page*)

Table 1a (*continued*)

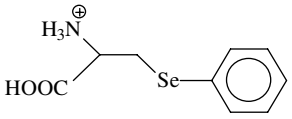
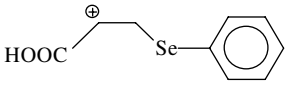
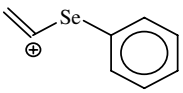
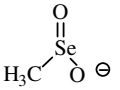
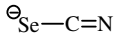
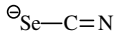
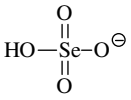
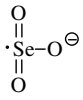
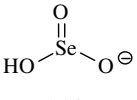
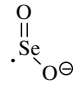
Analyte (molecular mass)	Precursor ion	MS^2 product ion	MS^3 product ion	MS^4 product ion
Phenyl-L-selenocysteine (244.15 amu)	 246	 229	 183	

Table 1b
MS/MS fragmentation pattern of the inorganic selenium species

Analyte (molecular mass)	Precursor ion	MS^2 product ion
Methaneseleninic acid (127.00 amu)	 127	 112
Potassium selenocyanate (144.08 amu)	 106	 106
Sodium selenate (188.90 amu)	 145	 128
Sodium selenite (172.94 amu)	 129	 112

2.3. Sample preparation

The supplements, commercialised as tablets or pills, were homogenised in a mortar. An aliquot (of the order of 0.01 g) was accurately weighed, added with 10.0 mL of methanol for the analysis of the organic species or with 10.0 mL of ultra pure water for the analysis of the inorganic species, sonicated for 10 min, centrifuged at 3500 rpm for 5 min and then 0.45 μm filtered. The solution was diluted 1/10 with the mobile phase at its initial ($t = 0$ min) composition.

For the ICP OES analysis amounts of the supplement ranging between 0.5 and 1.0 g were added with 5.0 mL of a 3:1 v/v mixture of 65% HNO_3 and 37% HCl and heated under stirring up to 200 °C. The residue was collected with 25.00 mL of ultra pure water, centrifuged, 0.45 μm, filtered and the solution brought to 50.00 mL in a volumetric flask.

2.4. Chromatographic conditions

For the separation of both organic and inorganic species, a stationary phase C18 Luna (150 mm × 2.0 mm, i.d., 3 μm, by Phenomenex, USA) was employed, equipped with a pre-column Security Guard Cartridge (4.0 × 2.0 mm, by Phenomenex, USA).

For the separation of organic species the mobile phase was a mixture of water and methanol both 0.05% in trifluoroacetic acid (TFA), eluting at a flow-rate of 0.2 mL/min under the following gradient conditions: $t = 0$ min: 0.05% TFA aqueous solution 90%; $t = 20$ min: 0.05% TFA aqueous solution 0%. The injection volume was 20.0 μL.

For the separation of inorganic species the mobile phase was a mixture of 1.0 mM TBA aqueous solution and methanol eluting at flow-rate of 0.2 mL/min under the following gradient conditions: $t = 0$ min: 1.0 mM TBA aqueous solution 90%; $t = 20$ min: 1.0 mM TBA aqueous solution 0%. The injection volume was 20.0 μL.

For the ion-interaction HPLC analysis of selenite in the absence and in the presence of ascorbic acid the stationary phase was a LichroCART Superspher RP-18e (250 mm × 4 mm × 5 μm) with a pre-column LiChrospher RP-18 (5 μm). The mobile phase was an aqueous solution of octylamine 5.0 mM, brought at pH = 6.4 for *o*-phosphoric acid, eluting at a flow-rate 1.0 mL/min. UV detection at 230 nm was employed.

In ion-chromatographic analysis, the stationary phase was a Dionex IonPac AS14A 4 × 250 mm Analytical, the mobile phase a mixture 50/50 v/v of 8.0 mM Na_2CO_3 and 1.0 mM $NaHCO_3$, flowing at 1.0 mL/min. The ionic suppression solution was a H_2SO_4 50.0 mM aqueous solution.

2.5. Mass spectrometry conditions for the organic species

The mass spectrometry experiments were conducted for the aminoacidic species by means of a Thermo "LCQ Duo" ion trap mass spectrometer. The ion trap mass analysis was performed in positive ion MS–MS mode. High purity nitrogen was used as nebuliser (operating pressure at 80 of the 0–100 arbitrary scale of the instrument for the sheath gas and 20 for the auxiliary gas). Helium (>99.999%) was the quenching agent. The ESI probe tip and capillary potential were set at 4.50 kV and +3.00 V,

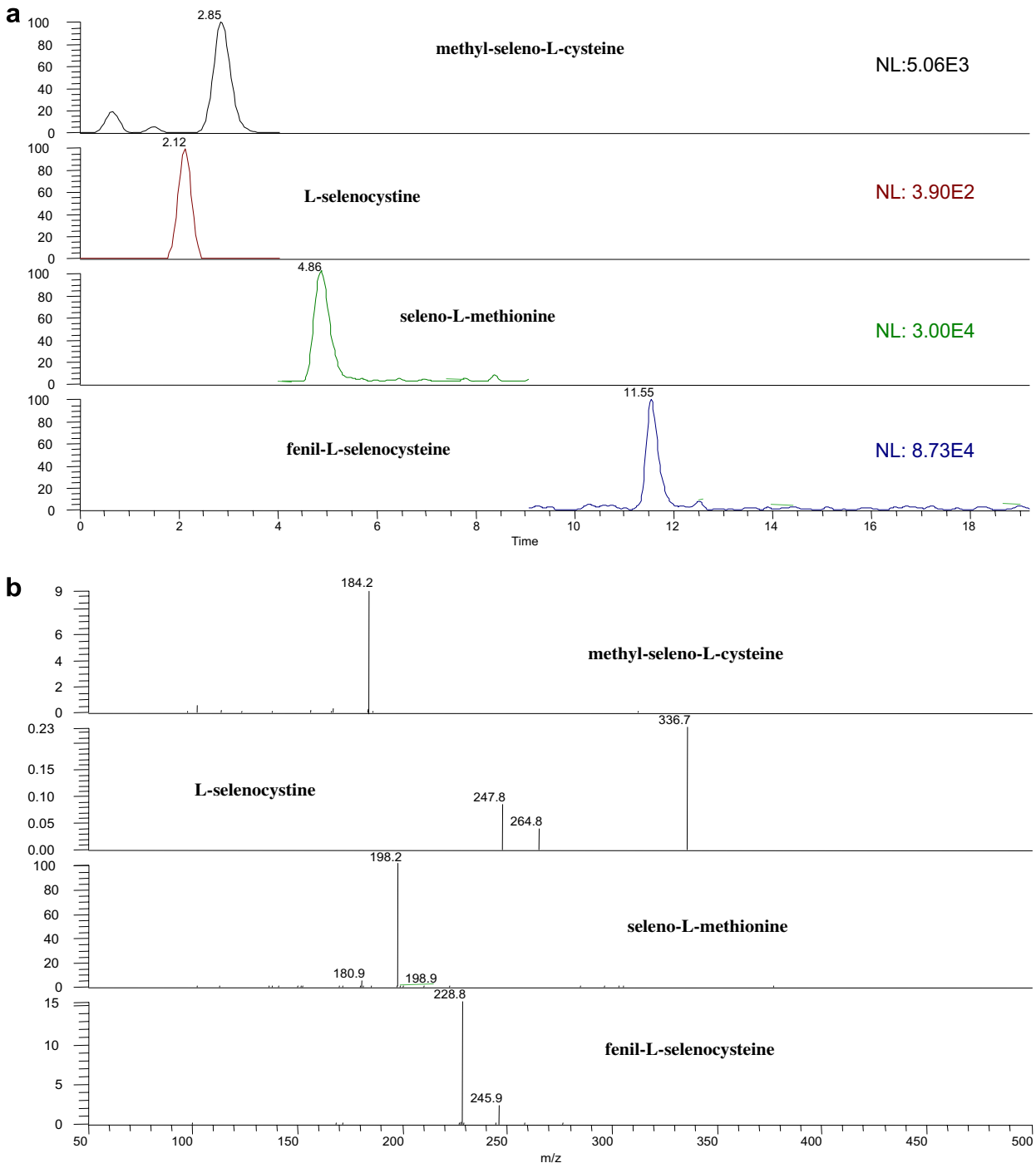


Fig. 1. Chromatographic separation (a) and mass spectra (b) of methyl-L-selenocysteine, L-selenocystine, seleno-L-methionine and phenyl-L-selenocysteine (100.0 μg/L each). Stationary phase C18 Luna (150 mm × 2.0 mm, i.d., 3 μm), pre-column Security Guard Cartridge (4.0 × 2.0 mm, by Phenomenex, USA). Mobile phase: water and methanol mixture, both 0.05% in trifluoroacetic acid (TFA) at flow-rate of 0.2 mL/min under the following gradient conditions: $t = 0$ min: 0.05% TFA aqueous solution 90%; $t = 20$ min: 0.05% TFA aqueous solution 0%. Injection volume 20.0 μL.

respectively. The heated capillary was set at 150 °C. Ion optics parameters were optimised to the following values: tube lens offset −5.00 V; first octapole voltage −0.75 V; inter octapole lens voltage −72.00 V; second octapole voltage −8.00 V. The mass to charge range was 50–300m/z. The mass spectrometer operated in positive ions (PI) MS–MS mode (three microscans, 200 ms inlet time). The conditions were optimised by tuning of the peak of methyl-seleno-L-cysteine at 184m/z, that corresponds to the molecular ion $[M + H]^+$.

2.6. Mass spectrometry conditions for the inorganic species

The mass spectrometry analyses of the inorganic species were performed by a Varian 1200 L triple quadrupole system equipped with an electrospray (ESI) ion source. The

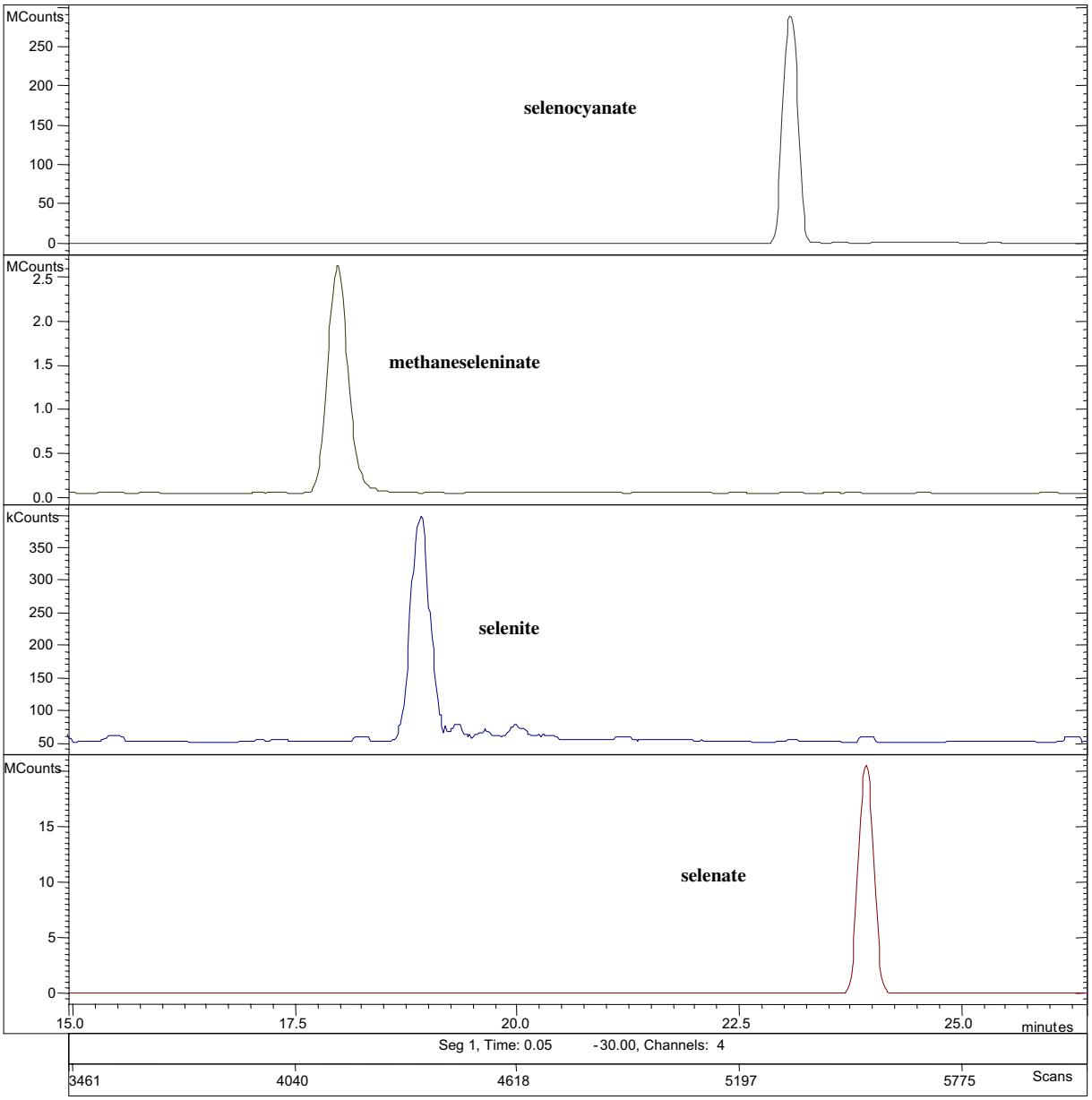


Fig. 2. Chromatographic separation of selenocyanate, methaneseleninate, selenite and selenate (100.0 μg/L each). Stationary phase C18 Luna (150 mm × 2.0 mm, i.d., 3 μm), pre-column Security Guard Cartridge (4.0 × 2.0 mm, by Phenomenex, USA). Mobile phase: 1.0 mM TBA aqueous solution and methanol mixture, eluting at flow-rate of 0.2 mL/min under the following gradient conditions: $t = 0$ min: 1.0 mM TBA aqueous solution 90%; $t = 20$ min: 1.0 mM TBA aqueous solution 0%. Injection volume was 20.0 μL.

ion optics parameters were the following: capillary temperature: 300 °C; shield voltage: 600 V; source voltage: −3.80 kV; capillary voltage: −4000 V; Q1 offset: 1.0 V; Q3 offset: 20.0 V. The mass analysis was carried out in negative ions mode (NI). Argon (>99.999%) was used as collision gas at 0.24 Pa pressure.

3. Results and discussion

3.1. Mass spectrometry characterisation of the organic and inorganic species

The mass spectrometry characterization was performed through the identification of the major product ions that form in the collisional sequential fragmentations of MS^n analysis for the organic compounds and MS–MS for the inorganic species: a direct infusion of the analytes was firstly performed. Solutions of the analytes, respectively in methanol (organic species) and ultra pure water (inorganic species), at concentration 10.0 mg/L (flow-rate 20.0 μL/min) were undergone to ESI source ionisation: in positive ions mode (PI) for organic species and in negative ions (NI) for the inorganic ones.

The ESI mass spectra (MS^n) recorded for the aminoacidic species show the presence of the carboxylic group (saturated by Na^+ ion) and of a protonable amino group. For each analyte a typical mass spectrum profile was identified and hypotheses were made about the structures of the most

Table 2
Quantitative analysis parameters

Species	Concentration range (μg/L)	LOD (μg/L)	LOQ (μg/L)
Methyl-seleno-L-cysteine	10.3–100.0	3.8	10.3
L-selenoystine	10.3–100.0	3.5	10.3
Seleno-L-methionine	11.0–100.0	3.4	11.0
Phenyl-L-selenocysteine	50.3–150.0	15.9	50.3
Methaneseleninic acid	4.0–100.0	0.8	4.0
Potassium selenocyanate	5.2–200.0	1.5	5.2
Sodium selenate	16.1–500.0	5.1	16.1
Sodium selenite	78.0–500.0	27.7	78.0

meaningful product ions. The results reported in Table 1a indicate that the behaviour of the seleno aminoacids considered is similar: the protonated aminic group gives a m/z signal that corresponds to the species $[M + H]^+$ and the MS/MS analysis shows the presence of the species $[M + H–NH_3]^+$, formed for loss of a neutral fragment of ammonia.

Both methanseleninate and selenite generate SeO_2^- ion, through the loss of a methyl or an hydroxyl radicals, respectively. SeO_3^- ion is in turn the principal fragment of selenate, which generates also smaller amount of SeO_2^- (Table 1b). No fragmentation is observed for selenocyanate, for which a precursor/precursor scan event was adopted.

A flow-injection analysis was then performed, in which a solution (5.0 mg/L) of each analyte was injected in the mass spectrometer in the presence of the mobile phase, in order to simulate chromatographic conditions and to evaluate the mass spectrometry response in the presence of the mobile phase. The response was similar to that obtained for direct infusion and indicates that the presence of the mobile phase does not significantly affect the results.

3.2. HPLC–MS/MS analysis

Figs. 1 and 2 show the chromatograms and the mass spectra, recorded under the optimised conditions already reported, respectively for the aminoacidic species (Fig. 1) and for the inorganic species (Fig. 2).

For each analyte a calibration plot was constructed reporting the peak area versus standard concentrations at four different concentration levels, by injecting the solution of the analyte at increasing concentrations, in order to overcome possible memory effects. Relative standard deviation (three experiments) in peak area is within 2.0% and reproducibility in retention times always lower than 3%. The linearity of the response was verified in the concentration ranges given in Table 2 and correlation coefficients R^2 were always greater than 0.9888.

Detection limit (LOD) values, expressed as the concentration of the analyte required to give a signal equal to the average background (S_{blank}) plus three times the standard deviation of the blank ($LOD = S_{blank} + 3S_{blank}$) (Miller and Miller, 2000), range between 0.8 and 27.7 μg/L. The quantification limits $LOQ = S_{blank} + 10S_{blank}$ are in the range between 4.0 and 78.0 μg/L (Table 2).

3.3. Analysis of commercial dietary supplements

Six typical commercial diet supplements, that do not contain yeast, identified as A–F, bought both in pharmacies and in supermarkets were analysed. Fig. 3 reports typical examples of the chromatogram and the mass spectra of the supplement A containing seleno-L-methionine (Fig. 3a) and of the supplement F, that contains selenate (Fig. 3b).

Table 3 reports the labelled composition, the total selenium content and the daily dose suggested, as given by the producer. Table 3 also reports the qualitative and quantitative composition we found by the HPLC–MS/MS methodology. To complete the analytical profile, the results of the ICP OES analysis of the mineralized samples are also reported.

For the supplement A, acquired in pharmacy, ICP analysis gives a total metal content very close to that labelled and also the speciation analysis roughly corresponds to that expected: the presence of seleno-L-methionine is confirmed, even if the amount is only about 75% of the declared.

As concerns the supplement B, bought in a food supermarket and that reports the presence of seleno-L-methionine, the selenium content evaluated by ICP OES spectrometry is only about 37% of that labelled and no seleno-L-methionine or any other amino acidic species has been found.

Particular attention must be paid to the supplement C, which is commercialised as naturally containing selenium and for which no indication is given about speciation and daily intake. The supplement contains relevant amounts of phenyl-L-selenocysteine and lower amounts of potassium selenocyanate. The total selenium content, as confirmed by ICP analysis is very high: this result, associated with the lack of a daily suggested dose and with the definition of "natural product", could easily mis-direct the consumer and lead risks of selenium overdose.

As concerns the analysis of the supplements that report the presence of selenate and selenite, large discrepancies are encountered between the data expected and those found. So for the supplements D and E (that are given to contain selenite), even if the ICP OES analysis gives values of total selenium comparable with those declared, the HPLC–MS analysis gives no presence of selenite, at least at the detection limit of the method. Furthermore, the mass to charge ratio characteristic of selenite is still not detectable even for suitable standard additions of selenite itself. To explain these results, the possibility of reactions taking place among the different components present in the supplement has been considered. According to literature, selenite is easily reduced to selenium by ascorbic acid (Lipinski, 2005). The formulations of the supplements D and E contain, besides selenite and many other components, ascorbic acid at concentration levels more than 1000 folds greater than selenite. To verify the possibility of redox reactions taking place between ascorbic acid and selenite, independent experiments based on negative ion LC–MS/MS were performed on a model solution containing selenite and ascorbic acid, in a 1/1000 concentration ratio. The analyses

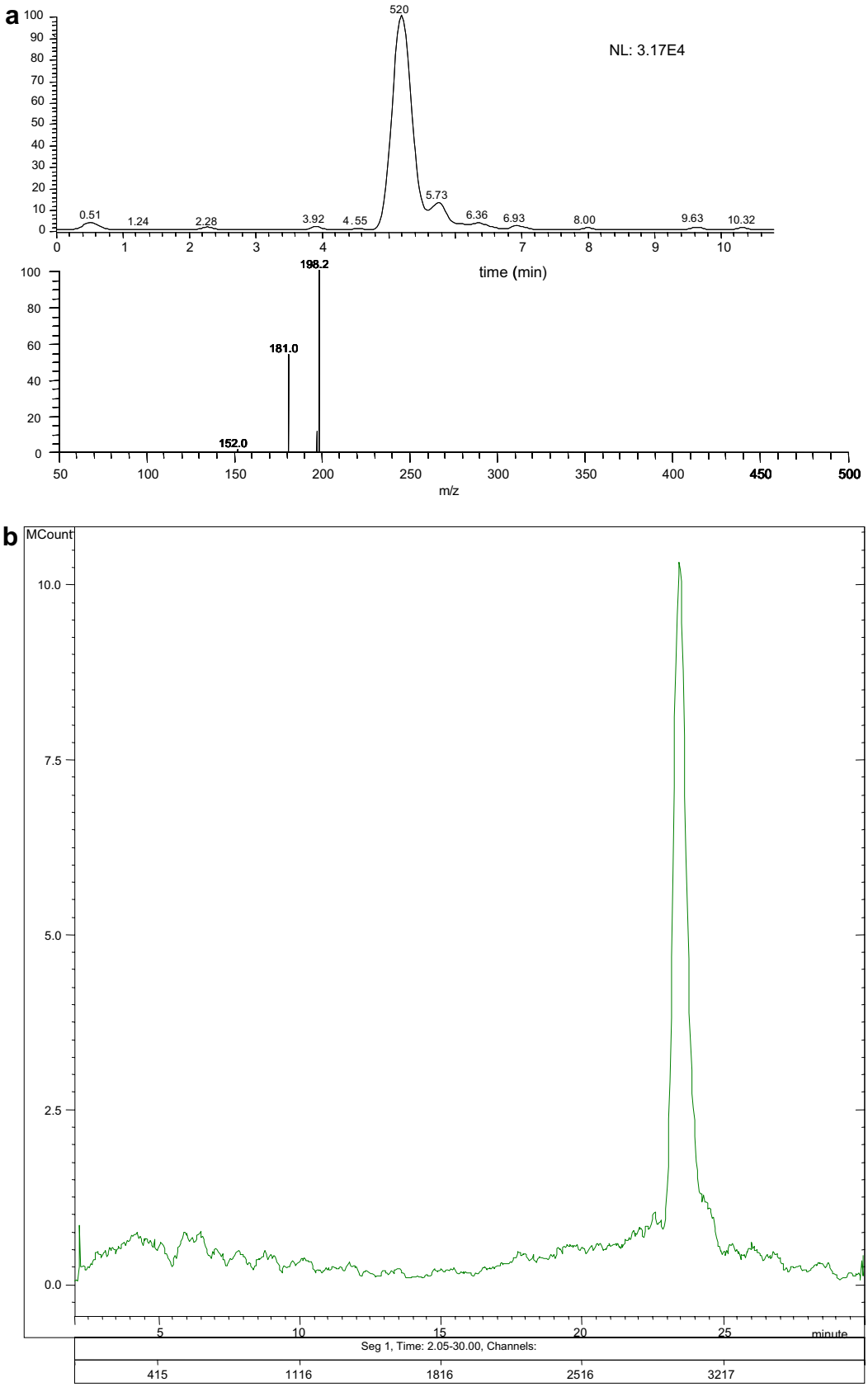


Fig. 3. Typical chromatograms and mass spectra of (a) a supplement containing seleno-L-methionine (supplement A in Table 3). Chromatographic conditions as in Fig. 1. (b) A supplement containing selenate (supplement F in Table 3). Chromatographic conditions as in Fig. 2.

Table 3
Results of the speciation analysis of eight commercial supplements

Supplements	Declared composition of supplements	Declared selenium amount, given as Se (mg/kg)	Suggested Daily Intake (μg)	LC–MS/MS (mg/kg)	LC–MS As total Se content (mg/kg)	ICP OES total Se (mg/kg)
A	Seleno-L-methionine	60.0	54	Seleno-L-methionine 107.6 ± 5.3	43.3 ± 2.8	58.0 ± 1.9
B	Seleno-L-methionine	62.5	50	Seleno-L-methionine not found	–	23.0 ± 2.1
C	Naturally containing selenium	Not given	Not given	Phenyl-L-selenocysteine 402.1 ± 18.9 KSeCN 2.1 ± 0.2	131.2 ± 3.3 (130.0 ± 7.4) from phenyl-L-selenocysteine and 1.2 ± 0.1 from KSeCN	183.0 ± 8.2
D	Sodium selenite	32	50	Not found	–	29.4 ± 1.8
E	Sodium selenite	12.5	50	Not found	–	20.1 ± 1.2
F	Sodium selenate	17.9	25	Na_2SeO_4 28.2 ± 3.7	12.0 ± 1.7	22.0 ± 2.0

showed that only after about 1 h from the preparation, the signal of selenite is no more present at detectable amounts. Eventually we also measured selenite by injecting the model solution immediately after mixing and we obtain only 18% of the initial concentration of selenite.

To support the results obtained in LC–MS/MS and to exclude that the effects observed are not due to mass signal suppression, independent experiments were performed, based on ion-interaction chromatography and UV detection at 230 nm, at which both ascorbic acid and selenite show appreciable absorbance. Model solutions containing ascorbic acid and selenite were prepared: in order to have a good resolution of the two chromatographic peaks the concentration (mg/L) ratio of ascorbic acid to selenite was 100/1 instead of 1000/1 as in the supplement. The results obtained in solutions 5.0 mg/L in selenite and 500.0 mg/L of ascorbic acid show that the peak of selenite after about 2 h from the preparation is present at percentages of about 50% of that expected and progressively decreases to practically disappear after 24 h.

As it concerns the supplement F, the label reports the presence of selenate and the spectroscopic analysis gives amount in total selenium even greater than that declared. But the results of HPLC–MS analysis give much lower amounts in selenate than those expected. Also the supplement F contains a great excess of ascorbic acid (molar ratio of ascorbic acid to selenate greater than 2500). On the basis of the redox potentials, the possibility that also selenate is reduced by ascorbic acid must be considered. To confirm this hypothesis, the ion-interaction HPLC–UV method is not useful, due to the low absorbance of selenate in the UV–Vis region. An ion-chromatographic method was then employed, based on conductometric detection. While the analysis of model solutions confirm the results previously obtained concerning the interaction between selenite and ascorbic acid, it was not possible to obtain information about the possible reduction of selenate, due to both low conductivity of selenate and the overlapping of the peaks of selenate and of dehydroascorbate (the oxidised form of ascorbate). But, by ionic chromatography it was possible to compare the decreasing of the concentration of ascorbic acid in two model solutions, containing ascorbic acid at concentration 100.0 mg/L in the absence and in the presence of selenate (at concentration 1.0 mg/L). In the absence of selenate the concentration of ascorbic acid decreases of about 18.4% in 24 h, while in its presence the concentration decrease is around 34.0%. These results support the hypothesis according which selenate and selenite are reduced partially or totally by the great excess of ascorbic acid.

It is impossible to say if the same process observed in standard solution takes place in the real samples and how the reactions depend on the aggregation status (solid or gel) under which the supplement is commercialised. But, as matter of fact, if reactions take place among the components, it is difficult to say under which speciation form selenium is assumed by the organism.

4. Conclusions

We believe that it is worth paying attention what these results suggest: side reactions are likely to occur among the different components of formulations. When analysing samples characterised by complex matrix, the possibility of side reactions taking place among components of a formulation or a food matrix must be considered and verified.

This possibility has also been recently evidenced in other studies concerning food dyes in beverages (Gosetti, Gianotti, Mazzucco, Polati, & Gennaro, 2007; Gosetti, Gianotti, Polati, & Gennaro, 2005) or food additives (Lau, McLean, Williams, & Howard, 2006).

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