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Review

Selective detection and identification of Se containing compounds—review and recent developments

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Abstract

The complexity of selenium (Se) chemistry in the environment and in living organisms presents broad analytical challenges. The selective qualitative and quantitative determination of particular species of this element is vital in order to understand selenium's metabolism and significance in biology, toxicology, clinical chemistry and nutrition. This calls for state-of-the-art analytical techniques such as hyphenated methods that are reviewed with particular emphasis on interfaced separation with element-selective detection and identification of the detected selenium compounds. Atomic spectral element specific detection for monitoring chromatographic eluent enabled quantitative determination of selenium species in selenized yeast and qualitative measurement for breath samples. Gas chromatography with atomic emission detection (AED) of ethylated species and fluoroacid ion pair HPLC applied to the analysis of currently produced or archived selenized yeast and *Brassica juncea* have revealed the presence of a previously unrecognised Se–S amino acid, *S*-(methylseleno)cysteine.

Keywords: Element-selective detection; Reviews; Detection, GC; Selenium-containing compounds

Contents

1. Introduction

Selenium (Se) is an essential element for animals and possibly for plants, but its physiological nature in humans is ambivalent since it can cause disease by deficiency, but is toxic at levels relatively close to those required for health.

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Analytical speciation is vital because the bioavailability and toxicity of an element depend on its chemical binding. Geologically, there are no substantive deposits of elemental selenium and it usually occurs in the sulfide ores of heavy metals deposited as a result of vulcanism and in some coal deposits. Selenates (SeO_4^{2-}) are easily leached from soils, transported to ground waters and most readily taken up by plants [1]. Selenites (SeO_3^{2-}) occur in mildly oxidizing neutral pH environments and are less soluble than selenates. Selenium pollution results from industrial activities such as oil refineries and coal-burning electric utilities [2]. Comprehensive discussion of environmental selenium is available in the texts authored by Frankenberger et al. [3,4].

Se accumulates in living tissues, the selenium content of human blood being about 200 ng/mL, 1000 times greater Se in surface waters. Biogeochemical cycling of Se in aquatic systems involves geological erosion and anthropogenic influences. It is incorporated as organoselenium compounds sequentially through plankton, lower and higher vertebrates or can be recycled through microorganisms. The chemical and biochemical transformations involved require analytical identification and quantification of selenium species at the highest sensitivity possible. Dimethyl selenide and dimethyl diselenide have been proposed as primary Se carrier species [5]. Se-accumulating plants belonging to the families *compositae*, *leguminosae*, *cruciferae* and *allium*, when grown in selenium-rich soils, may accumulate Se to concentrations of several thousands of mg/kg dry mass [6] and may be used to remove selenium from contaminated soils by phytoremediation [7]. Pyrzynska [8] has reviewed the determination of selenium species in environmental samples. Concentrations of selenite and selenate in environmental sea-water, ground water and fresh water samples have been reported to range from sub-ng/L in pristine conditions to hundreds of ng/L in contaminated sources; organoselenium levels have been found with similar ranges.

Speciation information is needed for the following fields. The elucidation of biogeochemical transformations of selenium compounds, the identification of natural and anthropogenic compounds with beneficial or detrimental effects, improvement in species-defined environmental remediation, and increasing knowledge about suitable methods to prevent or decrease toxicoses in plants, animals and humans.

Biologically, selenium is not bound by coordination, but forms covalent C–Se and Se–S bonds, Se-compounds being categorized as enzyme or gene products, although this distinction is not always complete. The former arises from enzymatic reactions such as reduction, methylation [9] and reactions leading to seleno-amino acid synthesis. Selenium is incorporated into gene products, according to the UGA codon that encodes for the selenocysteinyl residue [10]. To elucidate the complexities of selenium chemistry, the determination of *total element* although essential to determine element mass balance, provides insufficient information and must be accompanied by *speciation* of selenium [11].

Variation in the Se content of animal and vegetable-based foodstuffs is mainly due to geographical differences in the amounts and availability of soil Se as transferred to the food chain. Selenium-deficiency diseases are recognized in parts of China and elsewhere, but soils rich in Se (e.g. in excess of 5 ppm) are found in parts of the USA and other regions. The Se present in most plant-derived foods, notably as selenomethionine (SeMet) and selenocysteine (SeCys) derivatives, tends to have reasonably good bioavailability.

In the early 1970s, a discrete metabolic function was found for Se as an essential component of the enzyme glutathione peroxidase (GPX) [12] that participates in the antioxidant protection of cells by reducing hydroperoxides. Several Se-enzymes and selenoproteins are now recognized [13,14], and have been shown to contain Se in a highly specific form, selenocysteine. The nutritional essentiality of Se appears to be due to formation of the active selenol group of such SeCys-proteins, antioxidant protection by the GPXs, energy metabolism affected by the DIs and redox regulation of transcriptional factors and gene expression by the TRs.

Desirable Se dietary levels are in a relatively narrow range of consumption: food containing less than $0.1\,mg\,kg^{-1}$ will result in deficiency, whereas levels above $1\,mg\,kg^{-1}$ will lead to toxicity. Increasing attention has been paid to the speciation of Se in common foods. Plants metabolize inorganic Se anions, selenite and selenate to generate seleno-amino acids such as selenomethionine, selenocysteine, selenocysatathionine and methylselenocysteine. The *allium* family whose sulfur chemistry is well-defined exhibit parallel selenium chemistry and high-selenium garlic has found promise in mammary cancer prevention [15]. At present, no Se-enriched food or nutritional supplements have been fully characterized with respect to Se-compounds. This is of great importance since, with no standard of product identity, many different materials may be designated “Se-enriched” although they contain unknown amounts of different forms of Se that differ in both biopotency and/or anti-carcinogenic potential. A widely available selenium supplement is ‘selenized yeast’, which has been employed in a number of human nutritional clinical trials [16]. There is evidence that the pattern of Se-compounds in different Se-enriched products varies substantially.

Many epidemiological studies have pointed to an inverse association of Se status and risk to at least some cancers [17,61]. Most animal studies have shown that supranutritional levels of various Se-compounds can reduce the yields of tumors caused by chemical or viral carcinogens [17–20]. Clinical intervention trials in the USA [16,21,22] and China have shown lower cancer risks among subjects taking Se supplements compared to controls.

Evidence suggests that anti-tumorigenic activities can be supported by metabolites of forms of the element that naturally occur in foods: the Se-amino acids, SeMet and SeCys, and methylated Se-compounds such as Se-methyl-SeCys. With varying efficiencies, these species can be converted to a number of Se-metabolites, including methylselenol

(CH_3SeH) which appears to be a key anti-tumorigenic metabolite [23,24] These findings make the consideration of "Se status" important in the maintenance of overall health. In so doing, they raise questions about the parameters by which Se status should be defined, and the values of those parameters that should be taken as appropriate for the maintenance of good health. If robust analytical methods can be devised to determine CH_3SeH and/or other critical Se-metabolites in accessible tissues, then these would be valuable for monitoring the anti-tumorigenic efficacy of Se.

2. Analytical selenium speciation

Table 1 lists some of the classes and individual selenium compounds found in environmental and biological systems. For valid speciation, analytes must be separated in space or time prior to selenium specific detection. Spacial separation techniques include radioimmunological assays (RIA) for selenoproteins that offer very low detection limits but require the proteins to be isolated in amounts sufficient for antibody production [25]. The time-separated hyphenated techniques are based on the coupling of electrophoretic or chromatographic separation with an atomic spectrometric or other selenium specific measurement [11]. This coupling may be off-line, e.g. SDS-PAGE with instrumental neutron activation analysis (INAA), or on-line, e.g. HPLC–ICP-MS.

The determination of selenium species, including selenoproteins have been reviewed [11,26,27]. The complexity of analyte matrixes and the typically low level of selenium compounds present even in enriched samples makes speciation a challenging task but selenium specific detection is a powerful approach. High elemental sensitivity is mandatory, and quadrupole mass spectrometry with an atmospheric pressure ionization source, such as the inductively coupled plasma (ICP) has been successful for HPLC detection [28,29]. Significant isotopic overlap from $^{40}Ar_2^+$ on the most abundant isotope ^{80}Se (49.6%) often necessitates monitoring the less abundant isotopes ^{82}Se (8.6%) or ^{77}Se (7.6%). However inter-element isobaric interferences are less pronounced in HPLC–ICP-MS than for the determination of total selenium. Isobaric interferences that can be partially overcome with high-resolution mass spectrometers or dynamic reaction cell/collision cell technology.

The low concentrations of the target species represents a clear challenge; another comes from the need that sample pretreatment must not change the chemical forms of the target analytes, or must change them in a known and controllable fashion. For example if a 250 mg sample of 1200 mg Se/kg selenium-enriched yeast is taken up into 5 mL of solution, and target compounds are present at levels of 10% of the total concentration, then before separation, the concentration of selenium is 6 mg L^{-1}. If the separation procedure produces a dilution of 100, the concentration of material after separation is of the order of 60 μg/L (60 ppb).

3. HPLC modes for selenium speciation

Reversed-phase, paired ion reversed-phase, ion exchange and exclusion modes have been employed for selenium speciation. Pedersen and Larsen [29] used a polymer based strong anion exchange column to resolve selenite, selenate, selenomethionine and selenocysine at pH 8.5. They added 3% methanol to the mobile phase that enhanced the ICP-MS signal to give a ca. 1 μg/L detection limit for 100 μL injections. Gammelgaard et al. [30,31] used anion exchange chromatography on a Dionex AS11-HC column with 25 mM NaOH in 2% methanol mobile phase and ICP-MS detection to determine selenite in human urine after selenomethionine supplementation. Johansson et al. [32] described assisted on-line species conversion hydride generation atomic absorption spectrometry for speciation of selenate, selenite, selenomethionine and selenocystine in CRM 402 reference material and biological samples. Emteborg et al. [33,34] quantified selenium species in certified reference materials by anion exchange with direct injection nebulization ICP-AES and graphite furnace AAS. Sloth and Larsen showed argon-dimer and other mass interferences at *m/z* 74, 76, 78 and 80 were reduced by as much as five orders of magnitude with methane as dynamic reaction cell reactant for (DRC) ICP-MS [35]. Larsen et al. [36] achieved the

Table 1
Selenium species in environmental and biological systems

Inorganic species	
	Se(O)-(element), selenide – Se(−II), selenate – SeO_4^{2-}, selenite – SeO_3^{2-}
Simple organic species	
	Methylselenol (MeSeH), dimethylselenide (Me_2Se), dimethyldiselenide (Me_2Se_2)
	trimethylselenonium cation (Me_3Se^+), dimethylselenone (Me_2SeO_2), dimethylselenoxide (Me_2SeO), methylseleninic acid anion ($MeSe(O)O^-$
	dimethylselenosulfide (MeSSeMe), selenocyanate($SeCN^-$,), selenourea ($Se{=}C(NH_2)_2$
Amino acids and low molecular mass species	
	Selenomethionine, selenocysteine, selenocystine, Se-methylselenocysteine, selenocysteic acid, Se-methylselenomethionine, *S*-(methylseleno)cysteine, selenomethionine selenoxide hydrate, selenohomocysteine, γ-glutamyl-Se-methylselenocysteine, Se-adenosylselenohomocysteine, selenocholine, selenobetaine, selenoglutathione
Other compounds	
	Selenopeptides, selenoproteins, selenoenzymes, selenosugars, Se-metal metallothionines

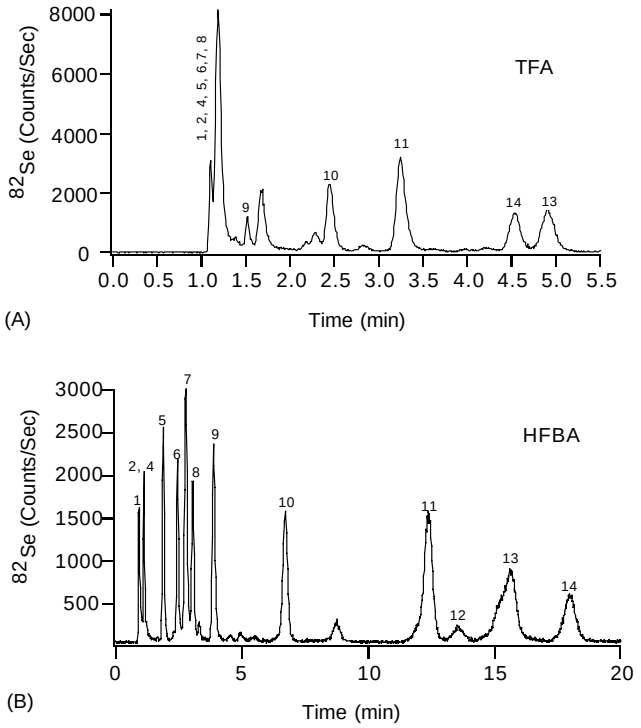

Fig. 1. Se-specific HPLC–ICP-MS chromatograms using (A) 0.1% TFA ion pairing agent and (B) 0.1% HFBA ion pairing agent. (1): Selenous acid (selenite); (2): selenic acid (selenate); (4): methylseleninic acid (methylseleninate); (5): selenolanthionine; (6): trimethyl selenonium cation; (7): selenocystine; (8): selenocystathionine; (9): Se-methylselenocysteine; (10): Se-2-propylylselenocysteine; (11): selenomethionine; (12): unknown; (13): γ-glutamyl-Se-methylselenocysteine; (14): Se-allylselenocysteine [40].

most comprehensive ion exchange separation of selenium species yet reported; cations were separated on a cation exchange column at pH 3 with a gradient of pyridinium formate and anions on an anion exchange column at pH 8.5 with a Tris salicylate mobile phase. Selenomethionine-Se-oxide was observed in selenized yeast and dimethylselenonium propionate (DMSeP) in an algal extract.

Selenoamino acids are too hydrophilic to be retained and separated with typical C_8 and C_{18} reversed-phase column conditions, but retention and separation is increased by using ion pairing reagents such as trifluoroacetic acid (TFA) [28,37] or octane sulfonic acid [38]. Perfluorinated carboxylic acids give better resolution and are effective for characterizing samples containing many different classes of organoselenium compounds [39,40]. Heptafluorobutanoic acid (HFBA) provides considerable resolution enhancement over TFA and allows separation of many additional organoselenium species (Fig. 1) [40]. The method also can determine the presence of selenoxides and organoanions. pH control at 2.0–2.5 enable cations such as trimethylselenonium and protonated seleno-amino acids and selenoxides to be resolved. Anionic species show some retention as protonated forms retained by reversed phase partition. McSheehey et al. [41] isolated fractions of selenized yeast extracts for on-line and off-line investigations by ICP-MS using semi-preparative reversed-phase columns for two-dimensional HPLC. Montes-Bayon et al. [42] speciated wild-type and genetically modified selenium-accumulating *Brassica juncea* with HPLC–ICP-MS and ES-MS detection and found a high preponderance of Se-methylselenocysteine being in the modified sample.

4. Detection of biomacromolecular selenium species by HPLC–ICP-MS

Lobinski et al. [26] have summarized applications of HPLC with selenium-specific detection for the analysis of biological samples with respect to column type, mobile phase and detection modes. Resolution of small compounds of similar molecular mass may be achieved in addition to the possibility of the detection of selenoproteins [43]. SEC–ICP-MS has had limited success for selenoproteins, the large dilution factor for Se limiting sensitivity in attempts to speciate glutathione peroxidase. SEC–HPLC was the first procedure to measure selenoprotein-P in human plasma, but it lacks the resolution and sensitivity for separation of the major Se-proteins. A human serum sample yielded three signals, but none co-eluted with the glutathione peroxidase activity [44]. Speciation of human breast milk whey gave four Se signals for apparent molecular weights of 15, 60, 1500 and >2000 kDa [45]. A combination of affinity chromatography with SEC–ICP-MS separated three major Se-containing proteins (albumin, glutathione peroxidase and selenoprotein-P) in human plasma [46].

Suzuki et al. [47,48] used HPLC–ICP-MS with ^{82}Se to study speciation of both endogenous and enriched selenium species. Selenium incorporation into cyanobacterial metallothionein induced under heavy metal stress was studied using SEC–ICP-MS, two pathways being indicated [49]. Selenite and selenate metabolism in rats was investigated [50] and exchange of endogenous and dietary selenium examined in brain, liver and kidney [51].

5. Chiral selenium speciation by HPLC–ICP-MS

The presence of asymmetric carbons in α-seleno-amino acids and related compounds produces chiral enantiomers with differing physiological activities. HPLC enantiomer separation is possible with various chiral stationary phases. Mendez et al. [52,53] speciated *D* and *L* selenomethionine isomers in yeast and parenteral solutions with a α-cyclodextrin stationary phase and also employed a teicoplanin-based chiral phase [54]. Sanz Medel and Gonzalez [55] compared hybrid chiral methodologies based on gas chromatography (GC), HPLC and capillary electrophoresis, coupled with ICP-MS. Sutton et al. [56] speciated enantiomers of *D*,*L*-selenocystine, *D*,*L*-selenomethionine and *D*,*L*-selenoethionine using a chiral crown ether stationary phase and examined a range of commercial dietary supple-

ments with ICP-MS detection. Ponce de Leon et al. [57] used this phase to separate nine seleno-amino acids in selenium enriched onion, were analyzed and some of the seleno-amino acid enantiomers were identified. Montes-Bayon et al. [58] used 1-fluoro-2,4-dinitrophenyl-5-*L* alanine amide to derivatize enantiomers of selenoamides for enhanced resolution.

6. GC microwave plasma atomic-emission detection (GC–AED)

Gas chromatography interfaced with atomic plasma emission spectroscopic detection (GC–AED) [59] has been used to detect and determine volatile organoselenium compounds present in or produced by plants. Headspace-GC–AED was used to detect selenium compounds in members of the *Allium* family such as garlic, elephant garlic, onion and broccoli (*Brassica*) [60]. Natural abundance organoselenium compounds in human breath after ingestion of garlic were identified using Tenax trap/cryogenic-GC–AED [61]. Calle-Guntinas et al. [62] reported absolute detection limits of 10 pg for dimethylselenide.

Free seleno-amino acids in normal and selenium-enriched samples have been determined by GC–AED of ethylated derivatives [61]. Calle-Guntinas et al. [63] compared AED, flame photometry and GC-MS detection for selective determination of selenomethionine in wheat samples.

Selenomethionine is the predominant seleno-amino acid in plants but selenocysteine is probably formed from glycine and selenite in mammalian tissues. Analytical approaches are typically based on degradation of the original matrix [39]. GC–AED and GC–MS provide a valuable alternative analytical approach to HPLC with good sensitivity. Seleno-amino acids have been derivatized with isopropylchloroformate and bis (*p*-methoxyphenyl) selenoxide [64], and with ethyl chloroformate [60,65]. Recent results from such investigations are described later. An alternative approach for selenomethionine involves quantitative formation of volatile methylselenocyanide with CNBr [66].

7. GC with ICP-MS detection

Mendez et al. [54] separated selenomethionine enantiomers as trifluoroacetyl-*O*-isopropyl derivatives by capillary GC–ICP-MS with a 1-valine-*tert*-butylamide modified chiral stationary phase, obtaining detection limits below 250 pg for each isomer. Bayon et al. [67] used a glow discharge (rf-GD) mass spectral ion source as a sample introductory technique for seleno-amino acids, detecting 100 pg of derivatized selenomethionine. Vonderheide et al. [68] used solid phase microextraction (SPME) for sample preparation followed by isopropylchloroformate derivatization for GC–ICP-MS obtaining sub-ppb detection limits.

8. Capillary zone electrophoresis (CZE) with ICP-MS detection

CZE can afford a high separatory efficiency and the possibility of analyzing for relatively labile species because of the absence of chromatographic packing, but ultrasensitive element-selective detection, such as high-resolution ICP-MS is needed because of the small sample amount injected. A commercial interface for CZE–ICP-MS is available which optimizes electrophoretic and nebulizer flows and has minimal dilution and sample consumption [69].

Michalke and coworkers [70–72] have identified and determined selenate-carrying glutathione (GSSeSG) and differentiated methionine, selenomethionine, cystamine and selenocystamine selenate and selenite in milk with speciation at the 10–50 $\mu g/SeL^{-1}$ level. Mounicou et al. [73] used a two-dimensional separation approach for selenized yeast speciation, based on size exclusion followed by CZE–ICP-MS, coupling to the ICP via a self-aspirating total consumption nebulizer. Detection limits of low molecular weight selenium species were in the range 7–18 μg/L, but problems were encountered with recovery of high molecular weight Se-species from the CZE capillary.

9. Identification of selenium species

HPLC–ICP-MS, GC–AED and CZE–ICP-MS have adequate sensitivity and selenium selectivity for selenium speciation in many samples but provide no structural identification. This lack becomes more problematic as more efficient separations are achieved. Chromatographic identification may be based on migration/retention time, but authentic standards of selenium compounds are often not available for retention-based identification. Approaches to analyte authentication involve preparation of additional synthetic standards or isolation of purified selenium compounds from sample matrixes followed by further characterization; e.g. by electrospray (ES-MS) [40,74,75,76] or matrix-assisted laser desorption ionization (MALDI-MS) [77].

Fan et al. [75] synthesized: Se-methylselenomethionine, Se-methylselenocysteine and dimethylselenonium propionate, and employed a combination of two-dimensional multinuclear NMR, electrospray MS and GC–MS methods to identify Se- GC–MS is sensitive but restricted to volatilizable species; for the non-volatilizable metabolites, there is often a discrepancy between the sensitivity of HPLC–MS techniques and that needed for speciation in real samples. Purification and preconcentration of Se-species is usually needed.

Higher resolution MS techniques such as ESI-triple quadrupole MS, ESI-MALDI-TOF and ESI-QTOF provide high precision and exact mass determination. ESI MS has been applied to preparations of Se-methylselenomethionine, Se-methylselenocysteine and dimethylselenonium propionate (precursors of volatile alkylselenides) [74].

Pneumatically assisted ESI MS was used to identify Se-adenosylhomocysteine in an extract of selenized yeast [78]. Kotrebai et al. [40,76] synthesized a number of seleno-amino acids and matched their retention times with signals produced by extracts of yeast supplements and garlic and plant extracts. McSheehy et al. [79,80] speciated selenium in garlic and yeast by parallel ICP-MS and electrospray tandem MS and by three-dimensional SEC–CZE–ICP-MS. Ogra et al. [81] identified a novel selenium metabolite, the selenosugar diasteriomer Se-methyl-*N*-acetylhexosamine in rat urine, by this method. Encinar et al. [82,83] identified water-soluble and triptic digests of yeast selenoproteins by HPLC–ICP-MS followed by MALDI-TOF and Q-TOF mass spectrometry.

10. Selenium speciation by complementary GC–AED and HPLC–ICP-MS

The value of applying parallel analytical separation and characterization procedures to validate more completely both qualitative and quantitative elemental speciation is clear, particularly when analytes are in low concentration and in complex matrixes. As discussed earlier, the use of gas chromatographic and liquid chromatographic separations can provide substantially more information and analytical credibility than can gained by either technique in isolation. Indeed such an approach represents good general analytical practice.

At present, despite substantial efforts, no Se-containing plant or animal components have been fully speciated nor have Se-enriched food or nutritional supplements have been fully characterized with respect to Se-compounds. There is evidence that the pattern of Se-compounds in different Se-enriched products varies substantially. While major Se-containing components have frequently been identified, typical quantitative selenium mass balance is incomplete when compared with total selenium content. Indeed 'minor' Se-compounds may prove to be critical in both biopotency and anti-carcinogenic potential This is of great importance since, with no standard of product identity, many different materials may be designated "Se-enriched" although they contain differing unknown amounts of different forms of Se. A widely available selenium supplement is 'selenized yeast', that has been employed in a number of human nutritional clinical trials [16]. Se speciation can vary substantially in such material.

The complexity of the selenium speciation profile is exemplified for a selenium-enriched yeast in Fig. 2. Heptafluorobutanoic acid paired ion reverse phase separation and ICP-MS detection were employed as in [40]. The chromatogram is displayed at both low and high (×25) sensitivity to emphasize both major components (in this case selenomethionine and selenomethionine selenoxide hydrate) but also to show the high complexity of minor (mostly unidentified) selenium species. From the standpoint of speciation of selenium compounds with respect to nutritional and biological activity it is an open question whether essential human selenium uptake is species dependent. In commercial nutritional supplements such as high selenium-enriched yeast selenomethionine or selenite are alternatively the major selenium species present. In the 'Clark trial' the former supplementation mode was used [16].

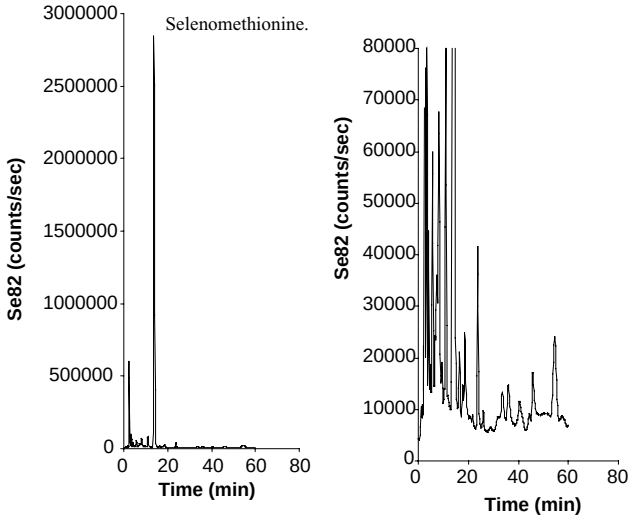

Fig. 2. Se-specific HPLC-ICP-MS chromatograms of enzymatic hydrolysis of 1250 ppm Se-enriched yeast using 0.1% HFBA ion pairing agent (right scale expanded).

As was noted above, such species can be converted to various Se-metabolites, including methylselenol (CH_3SeH) which appears to be a key anti-tumorigenic metabolite [23,24]. The question of whether other 'more potent' selenium compounds albeit present at lower levels in foods or nutritional supplements may be responsible for bionutritional activity depends on the prior identification of these 'compounds.

We have recently noted the presence of a previously unrecognized seleno-amino acid, *S*-(methylseleno)cysteine, that is present in selenium-enriched yeast that has been stored for a considerable time or subjected to elevated temperature. Selenium speciation by complementary GC–AED and HPLC–ICP-MS has proved particularly useful in identifying this Se–S species. [84,85]. The former technique has the distinct advantage of simultaneous detection of S and Se in the same chromatogram. Fig. 3 shows segments of selenium and sulfur capillary gas chromatograms of seleno-amino acids and analagous sulfur acids respectively, derivatized by ethyl chloroformate. GC–AED equipment and conditions are as reported previously [84]. Fig. 4 depicts selenium specific GC–AED of ethylated enzymatic hydrolyzates of supplemental enriched yeast tablets both as employed and archived from the 'Clark trial' and as presently manufactured. The profiles for the yeast tablets are virtually identical to those seen also for the free yeast powder and the species, identified as *S*-(methylseleno)cysteine is evident [84]. This important observation suggests that the nutritional tablet format

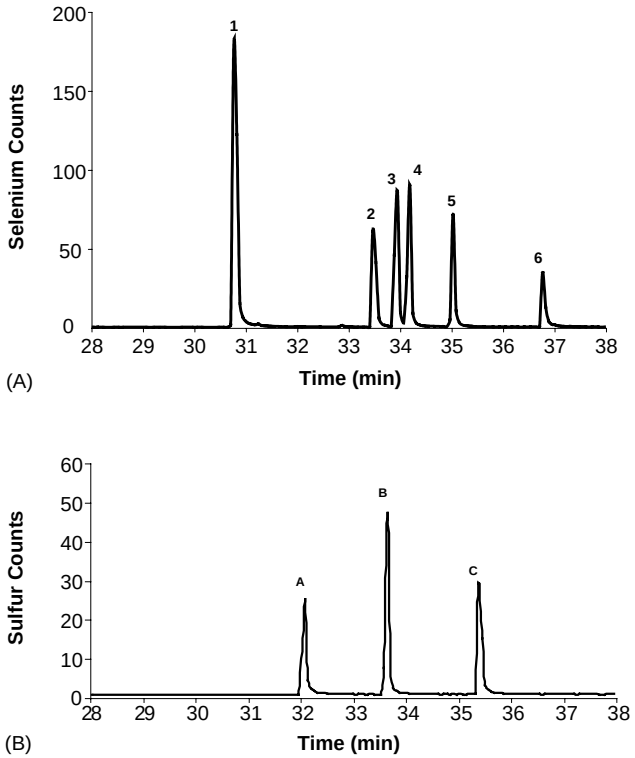

Fig. 3. Se-specific GC–AED chromatograms of ethylated amino acids. (A) seleno-amino acids: (1) Se-methylselenocysteine derivative; (2) selenomethionine derivative; (3) Se-allylselenocysteine derivative; (4) Se-propylselenocysteine derivative; (5) selenoethionine derivative; (6) selenocysteine derivative. (B) sulfuramino acids: (A) methionine derivative; (B) ethionine derivative; (C) cysteine derivative.

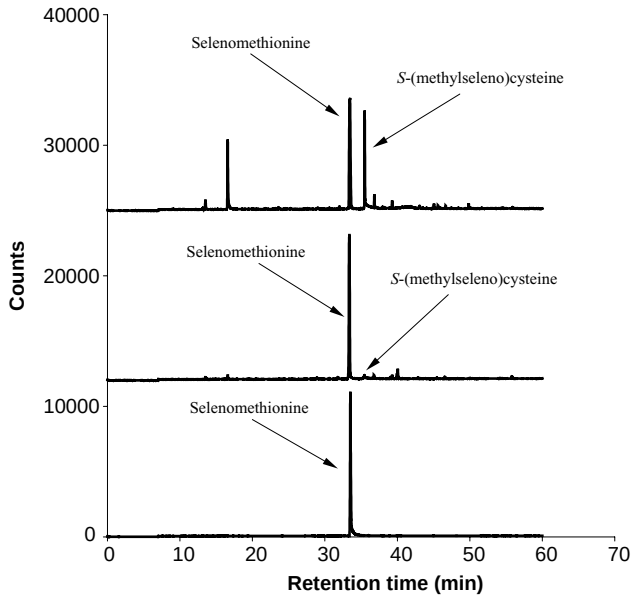

Fig. 4. Se-specific GC–AED temperature programmed chromatograms (Se 196 nm) of ethylated enzymatic hydrolyzates of archived (Clark trial [16]) 200 μg Se tablets (top), reference 200 μg Se tablets (center), and selenomethionine (bottom). Peak at ca.36 min contains sulfur.

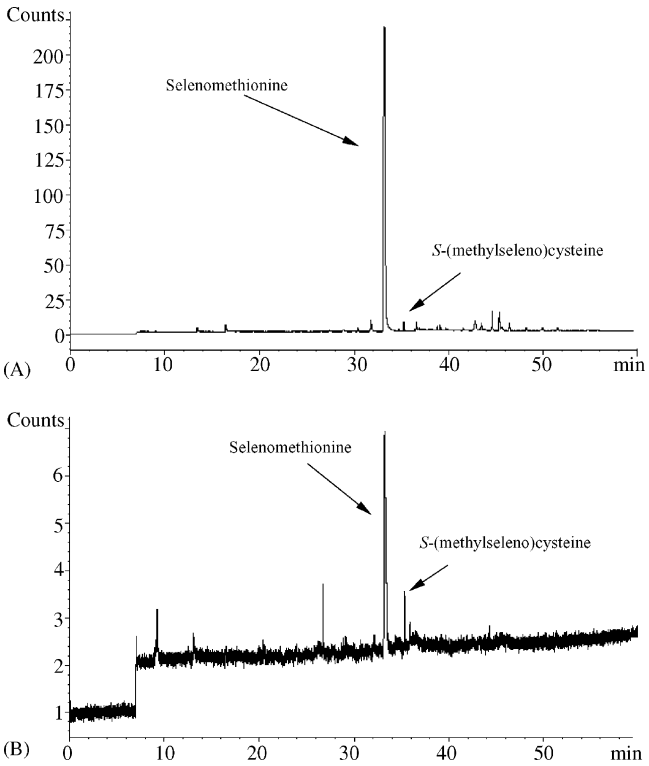

Fig. 5. Se-specific GC–AED chromatograms of ethylated amino acids from: (A) selenium enriched yeast; (B) selenite treated. *B. juncea* shoots.

retains the same speciation as the constituent yeast despite the presence of formulation excipients.

B. juncea is a selenium-accumulating plant that has received speciation attention, for example genetically modified plants being found to contain Se-methylselenocysteine [42]. In the course of a study of the effect of selenite or selenate on the Se speciation of *B. juncea* the presence of *S*-(methylseleno)cysteine has also been seen. Fig. 5 shows comparative GC–AED chromatograms of ethylated enzymatic extracts of a typical standard reference selenized yeast which does contain a small amount of *S*-(methylseleno)cysteine, and *B. juncea* shoots grown in a selenite growth medium. In which the Se–S acid appears at a higher relative level to selenomethionine. Se-methylselenocysteine is not apparent in these shoots. Fig. 6 shows a parallel HPLC–ICP-MS chromatogram. In view of this observation in this case, *S*-(methylseleno)cysteine may be present in different natural or enriched selenium materials. Studies as to whether this compound has nutritional of biological function are appropriate.

11. Anticipated developments

Problems in speciation of environmentally and biologically important organoselenium compounds remain challenging. Much has been achieved in terms of the detection

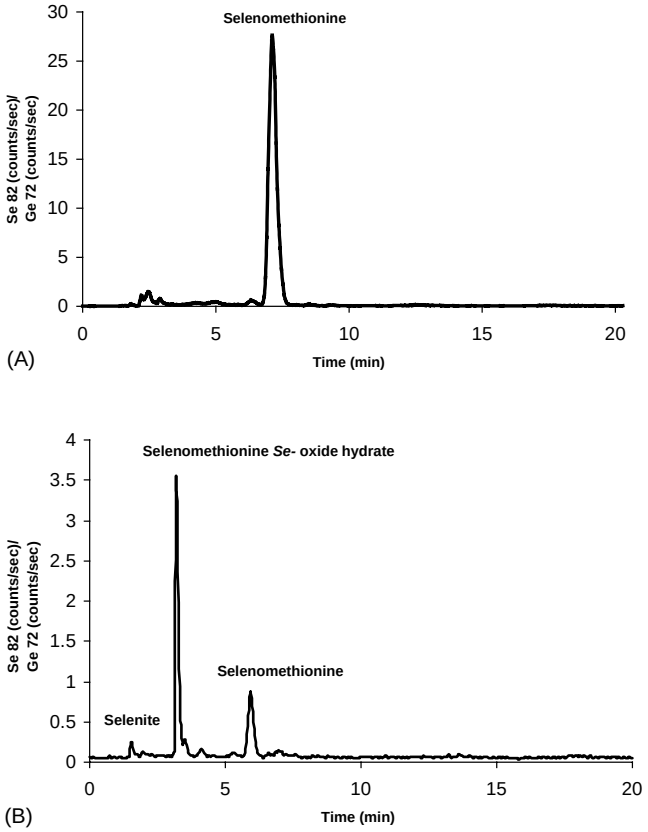

Fig. 6. Se-specific HPLC–ICP-MS chromatograms of enzymatic hydrolysis of: (A) selenium enriched yeast; (B) selenite treated *B. juncea* shoots.

in complex matrices, but the very low analyte levels often make the identifications problematic. The wider use of techniques that give direct access to structural information such as NMR and high-resolution mass spectrometry is important. The classical approach of concentration, purification and isolation of the seleno species detected can overcome the marginal sensitivity and vulnerability to matrix interferences that is often experienced with these techniques. Specific chemical derivative transformation of analyte compounds is a powerful approach that nevertheless requires careful control if speciation is not to be changed. More definitive results are needed such that barriers to a molecular-level understanding of Se clinical chemistry, ecotoxicology and nutrition can be eliminated.

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