ARTICLE

Understanding the role of Se in health using mass spectrometry

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Introduction

This article provides an overview of the mass spectrometry strategies being used for characterisation of selenium dietary supplements, in terms of the quantification and identification of selenium compounds, with special reference to selenised yeast. Most applications in this field reveal the versatility and potential of using a combination of inorganic and organic mass spectrometry with chromatography for selenium speciation analysis. We also describe current knowledge of the role and importance to human health of selenium and its compounds in the diet.

Selenium (Se), which is found naturally in a variety of foodstuffs, has long been recognised to have both toxic and beneficial effects. There is a fine balance between toxic and beneficial effects, which depends on the concentration and the form in which the element is present (Se species). The identification and measurement of these compounds is essential for medical and nutritional research into the use of selenium-containing drugs or supplements, which are widely commercialised as can be seen by visiting any health food shop. Reliable information on the amount and identity of the Se compounds present in food and food supplements will help to progress our understanding of the roles that Se supplementation in the diet play in reducing the risk of cancer and other human diseases. It will also help in the development of safe and effective products, and for future regulation of their production and use.

> "With what I hope is a new and correct perespective of the toxic mechanism of Se compounds, perhaps a way can be found to selectively use catalytic Se compounds to target metastatic cancer cells, drug resistant bacteria, and yes, even the HIV virus. This eventual possibility with Se-generating free radical pharmaceuticals is on the horizon… Such applied compounds could truly be magic 'free radical' bullets, perhaps one day even exceeding the efficacy of present day antibiotics and chemotherapeutic cancer drugs."
>
> J.E. Spallholz, 1994

Se and health

Selenium was described as toxic as far back as the 13th Century, by Marco Polo in China. Typical toxicity symptoms include hair loss, damaged nails, garlic breath, skin lesions, mottled pitted teeth, nausea and vomiting. Paradoxically, the existence of selenium-deficiency diseases in animals has also been understood for many years. These include white muscle disease, impaired reproduction, liver necrosis, exudative diathesis (damage to capillary walls) and pancreatic degeneration. More recently, several selenium-deficiency diseases in humans have been identified including Keshan disease (cardiomyopathy—wasting of the heart muscle) and Kashin–Beck disease (osteoarthropathy). Since the 1950s, the unique role of selenium in biological systems has come into focus. Se is the only trace element specified in the genetic code, as selenocysteine (SeCys) that is inserted into proteins to give selenoproteins. It is now recognised that adequate Se levels may be important for reasons as diverse as antioxidant defence, thyroid function, the immune system, antiviral effects, fertility and reproduction. In addition, there is a growing body of opinion that it may also reduce cancer risk.[1]

A range of inorganic and organic selenium compounds present in the human diet is shown in Table 1. The toxicity and potential beneficial effects of these compounds vary enormously. It is generally accepted that inorganic forms of selenium are more acutely toxic than organic forms. A common source of organic selenium for dietary supplements is Se-yeast. A number of human intervention studies show that chronic administration of Se-yeast provides no evidence of toxicity. Acute toxicity studies with Se-yeast have been carried out on rats, demonstrating that Se-yeast is considerably less acutely toxic than sodium selenite.

Only limited information is available regarding the beneficial effects of specific organic selenium compounds, although there seems to be a growing view that selenomethionine (SeMet) is a key selenium species for anti-cancer applications. Several studies on the correlation of the chemical form and metabolism of different selenium compounds with their anti-carcinogenic activity concluded that those compounds that are able to generate a steady stream of methylated metabolites, particularly the monomethylated species, are likely to have good chemo-preventive potential.[2] However, the relationship and mechanism by which Se supplementa-

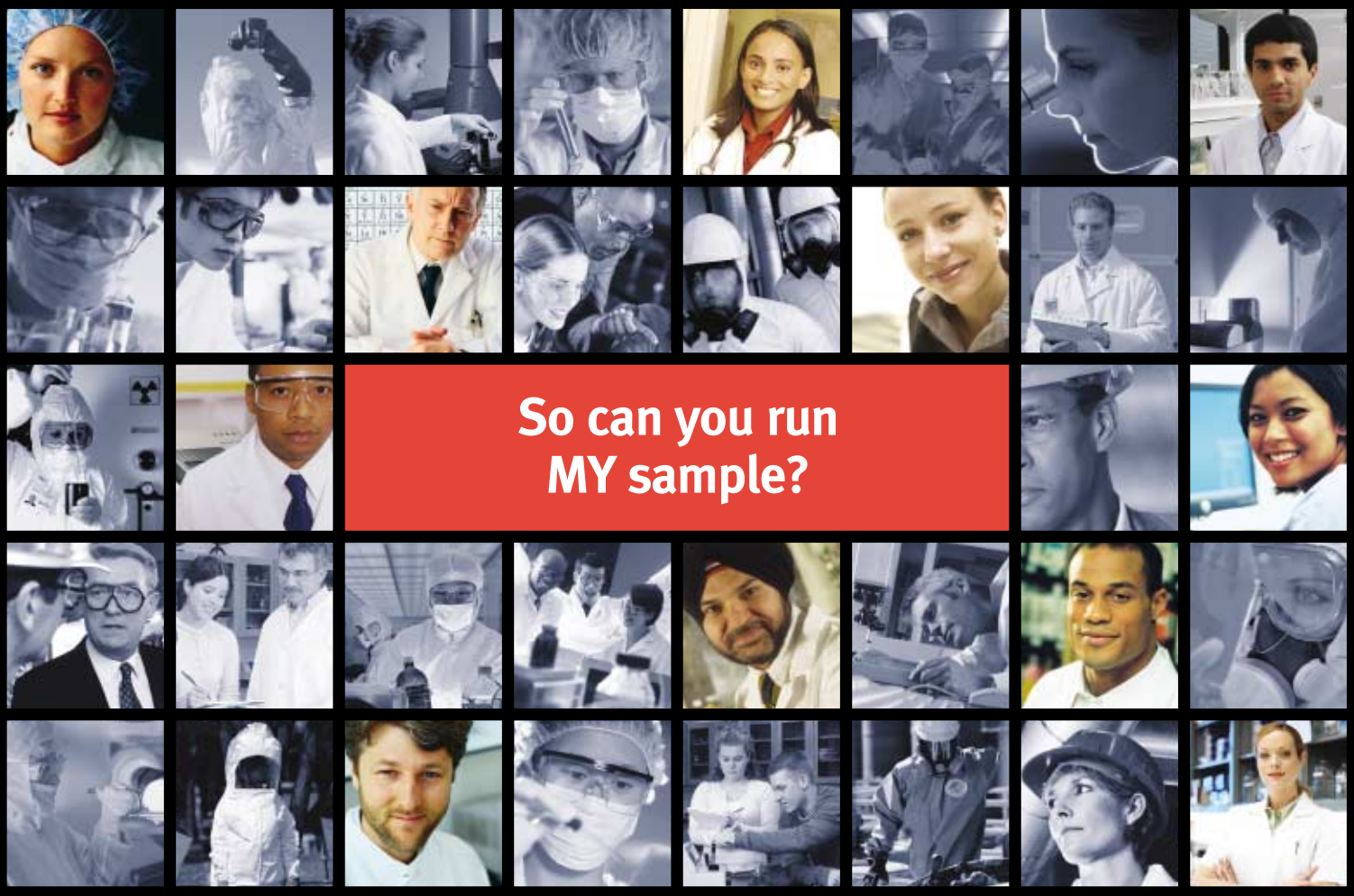

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Table 1. Inorganic and organic target Se-compounds in food and food supplements.

Selenite	SeO_3^{2-}
Selenate	SeO_4^{2-}
Selenomethionine*	$CH_3SeCH_2CH_2CH(NH_2)COOH$
Se-methylselenocysteine*	$CH_3SeCH_2CH(NH_2)COOH$
γ-glutamyl-Se-methylselenocysteine*	$NH_2(COOH)CHCH_2CH_2CONHCH(COOH)CH_2SeCH_3$
Se-adenosylselenohomocysteine*	$NH_2CH(COOH)CH_2CH_2SeCH_2C_4H_5O_3C_5N_4NH_2$
Selenocystine	$COOH(NH_2)CHCH_2SeSeCH_2CH(NH_2)COOH$
Selenocysteine	$HSeCH_2CH(NH_2)COOH$
Selenocystathionine*	$COOH(NH_2)CHCH_2SeCH_2CH_2CH(NH_2)COOH$

*identified in selenised yeast using hyphenated MS techniques

tion may reduce certain cancer risks is not fully understood. This is in part due to the lack of information on Se speciation, i.e. the concentration and identity of the chemical species in which Se occurs, in food and supplements.

Dietary sources and human intake

Intake of selenium from the normal human diet has been shown to vary widely between countries or regions and also over time, depending on both agricultural practice and dietary preferences. The highest mean concentrations of selenium (in all forms) are found in offal, fish, broccoli, *Allium* plants, nuts and eggs, with further contributions from other meat products, bread, miscellaneous cereals, poultry and milk. In the UK these foods account for approximately 70% of UK daily intake, which is around 30–40 µg/d. This is well below the recommended level for both the UK and US (55–75 µg/d) and also below the daily intake elsewhere (e.g. 55 µg/d in Belgium, 98 µg/d in USA and 113 µg/d in Finland). The UK intake has reduced significantly in recent years due to a drop in imports of high-protein, high-Se North American wheat since joining the European Union (EU). This has come about because changes in bread-making technology allow use of low-protein, low-Se EU and UK varieties. Reduced atmospheric deposition from fossil-fuel burning and higher grain yields have also reduced the Se concentration in the plant.

Broadly speaking, foods of animal origin contain selenium as Se-cysteine whereas foods of plant origin contain SeMet. However, metabolism by organisms means inorganic selenium and other species will exist in foodstuffs (e.g. Se-glutathione and Se-glutadithionine). There is little reliable data on the form which selenium takes in the various naturally occurring dietary sources, particularly for the metabolites. The lack of adequate methods to identify and measure organic selenium compounds at the low concentrations involved is a key factor in this respect.

In the absence of adequate natural dietary levels of selenium, there is a growing demand for food supplements. Selenium supplements commercially available include inorganic forms (sodium selenite, sodium hydrogen selenite and sodium selenate) and several organic forms (selenomethionine, selenium-methyl-Se-cysteine (SeMC) and Se-enriched yeast[3]). Selenised yeast has probably been the most widely investigated Se food supplement.[3] It is the only form of Se to date to have shown efficacy as an intervention agent in human cancer prevention studies.[3–5] Se-yeast is attractive as a supplementary source of selenium owing to its low cost, its ability to act as a precursor for selenoprotein synthesis and its high content of SeMet, a highly bioavailable form of Se found in most foods.

The variability of Se-yeast with respect to the amount and speciation of the Se, together with a lack of knowledge of the identity of the Se species that occur in this food supplement, remain a problem. There is, therefore, an urgent need to characterise and quantify the Se-species present in selenised yeast. This calls for state-of-the-art analytical techniques such as hyphenated mass spectrometry methods, which are based on the combination of a powerful separation technique with both element-selective and molecule-specific detection of the selenium compounds. It is also important to develop methods able to ensure correct identification and tracking of the Se species during food or supplement production and, for nutritional or clinical research purposes, during digestion, absorption and utilisation of the selenium in the body.

Modern trends in the analysis of Se compounds in high-Se yeast by hyphenated MS

The literature on the selenium speciation analysis of selenised yeast is abundant (see Figure 1) and has been reviewed.[6–9] The analytical methods used have been based on the coupling of a chromatographic separation technique with Se-specific detection, usually by inductively coupled plasma mass spectrometry (ICP-MS). Commonly used separation techniques include liquid chromatography (usually size-exclusion followed by ion-exchange, reversed-phase and ion-pair reversed-phase HPLC),[10–12] gas chromatography,[13] capillary electrophoresis[14] and 2D gel electrophoresis (for Se-containing proteins).[15] In some cases, electrospray ionisation (ESI) MS and matrix-induced laser desorption ionisation (MALDI) MS have been used for on-line molecular mass determination of selenium compounds.[16,17] However, with no fragmentation of the molecular ion possible, identification is often ambigu-

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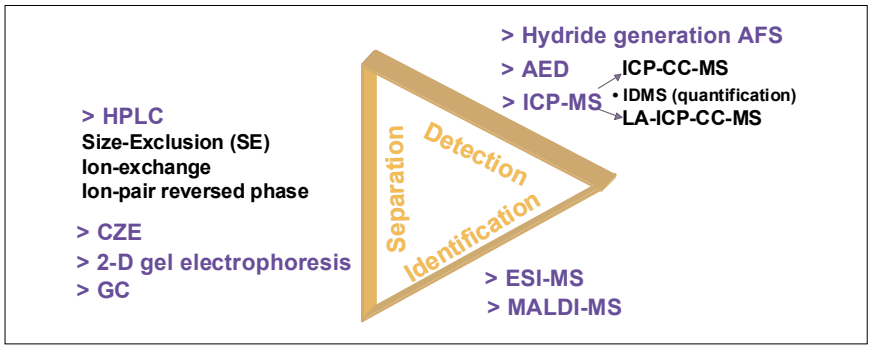

Figure 1. Analytical methods for the determination and identification of selenium compounds.

ous. MS^n using direct analyte infusion or on-line liquid chromatography has been used for confirmation and/or identification of some Se-compounds, including selenoamino acids[18,19] and peptide species,[20,21] in Se-yeast (see Table 1 for some organic selenium compounds, which have been identified in yeast). GC-MS has been used for molecular mass determination of Se-amino acids after their extraction from yeast and derivatisation.[22] Most studies reveal the versatility and potential of mass spectrometric detection in this field. Although great advances have been achieved, further developments are required. Despite a large number of publications, solutions to a number of analytical problems are still required. Those are summarised below.

A key outstanding challenge is the preservation of the identity of the selenium compounds during their extraction from the solid matrix prior to their analysis using chromatography and mass spectrometry. Numerous extraction techniques have been developed. The literature has shown a clear trade-off between selenium extraction efficiency and preservation of the compounds identity. Figure 2 shows a comparison, in terms of selenium extraction efficiency, between different sample preparation methods used for selenium speciation purposes. The most frequently used, being based on enzymatic hydrolysis, have been reported to extract about 60–80% of total Se present (mostly as SeMet) in Se-yeast. The extraction efficiency of enzymatic methods of hydrolysis critically depends on the type of enzyme and the extraction conditions (e.g. incubation time and sample to enzymes ratio), as recently reviewed by Yang *et al.*[23] The use of proteolytic enzymes for species liberation mimics the physiological conditions in the human intestine. Therefore enzymatic digestion methods are rather preferred than the harsher chemical hydrolysis with acids for speciation purposes, although the Se extraction efficiency shown by both procedures seems to be comparable (see Figure 2). However, the real efficiency of these methods is generally unknown and results obtained in different laboratories are often not comparable. This is due in part to the lack of matrix certified reference materials (CRMs) with information about Se species concentration and identity, for use in method validation. For extraction of the intact selenium compounds from the solid sample prior to their measurement and identification using hyphenated MS techniques, leaching the yeast with water has been used[10,11,19,20] in spite of the low Se extraction efficiency achieved (10–15% of the total Se in yeast) with this method.

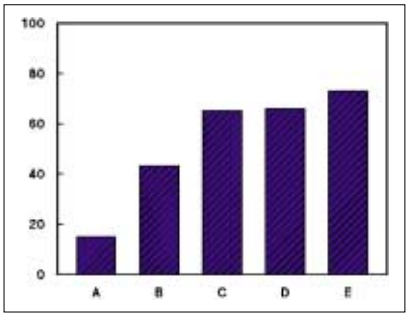

Figure 2. Extraction efficiency of selenium from selenised yeast as a result of various extraction/digestion methods. A: leaching with water; B: protein degradation with cyanogen bromide; C: hydrolysis with enzymes; D: hydrolysis with methanesulfonic acid and E: hydrolysis with hydrochloric acid.

There is an increasing interest in the use of isotope dilution mass spectrometry (IDMS) techniques for validation of methodologies for Se speciation analysis and for the accurate determination of Se-compounds, particularly for the certification of "speciated" matrix reference materials such as Se-yeast. Species-specific IDMS has recently been used in combination with collision cell ICP-MS and HPLC[24] or GC[25] separations for the accurate determination of SeMet in yeast. GC-IDMS was successfully used for the simultaneous determination of SeMet and methionine (Met) using ^{13}C-enriched SeMet (available from Sigma-Aldrich Company Ltd.) and Met spikes.[26] However, for Se species other than SeMet this remains a difficult task due to the lack of commercially available isotopically-enriched Se-containing species to use as the IDMS "spike".

Finally, information on the identity of the molecules incorporating or binding selenium in selenised yeast is still scarce because of the complexity of the system, the low concentration of the Se-compounds, and the lack of suitable reference standards to confirm their identification. Although the major component of Se-yeast has been identified as SeMet, previous speciation studies have indicated that this food supplement is probably a source of other organo-selenium compounds, some of which may have more potent anti-carcinogenic properties than SeMet. Further studies are needed to verify the presence of these species in the complex yeast sample and to perform accurate quantification of these compounds.

Novel MS strategies to measure and identify methyl Se compounds in yeast

For the reasons noted above, our work has focused on the development of novel hyphenated mass spectrometry methods for the characterisation of high-selenium yeast. Successful analytical methods will be essential to the characterisation of yeast "speciated" CRMs.

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On-line identification of the main compound present as SeMet was achieved using both inorganic and organic mass spectrometry, respectively ion-pair reversed-phase chromatography on-line with ICP-MS and ESI-tandem MS.[19] As a first step, SeMet in yeast enzymatic digests was identified by HPLC-ICP-MS, on the basis of comparison of retention times with a matching standard. Figure 3 shows the ^{82}Se profile obtained with this method for a yeast enzymatic hydrolysate; the fraction at t_R = 10.7 min) corresponds with the retention time of the SeMet standard. In order to verify the identity of this peak, analysis of the extract using ion-pair reversed-phase chromatography with on-line molecule-specific organic mass spectrometry was undertaken.

On-line molecular mass determination of the compound with t_R = 10.7 min was achieved using HPLC-ESI-MS. The observed Se isotopic pattern of the mass spectrum taken as the sum of the whole SeMet peak is shown in Figure 4(a), to be compared with the theoretical Se isotopic pattern (not shown), which was calculated on the basis of the known structural composition of SeMet. The two Se isotopic patterns are identical and centred at *m/z* 198 for the $[M+H]^+$ ^{80}Se ions. Collision induced dissociation was used to obtain the product ion spectrum of the *m/z* 198 precursor ion for the SeMet peak. The results, as presented in Figure 4(b), are consistent with the presence of SeMet in the yeast digests.

The use of a similar approach has for the first time enabled Se-methylselenocysteine (SeMC) and its carrier γ-glutamyl-Se-methylselenocysteine (see Table 1) to be identified in yeast extracts.[19,27] Mass spectral confirmation of the presence of methyl-selenocysteine and gamma-glutamyl-methyl-selenocysteine in yeast is of interest as these species appear to be metabolised in animals and, possibly, humans to methylselenol, an anti-carcinogenic Se-metabolite.

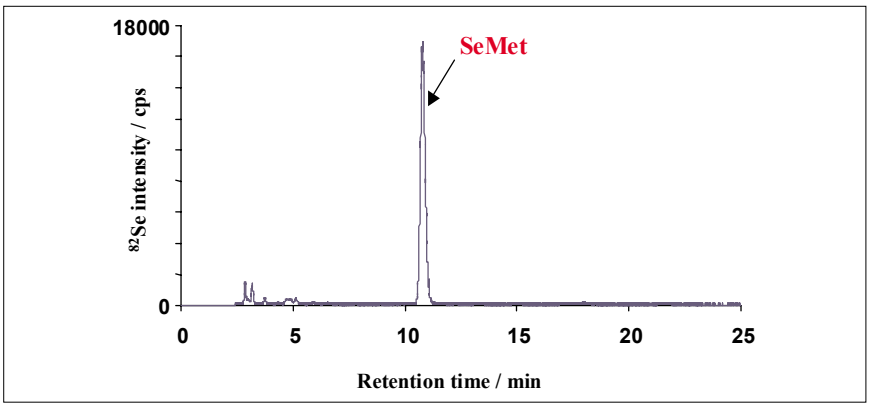

Figure 3. Elution profile of Se by ion-pair reversed-phase HPLC-ICP-MS for an enzymatic hydrolysate of Se-yeast.

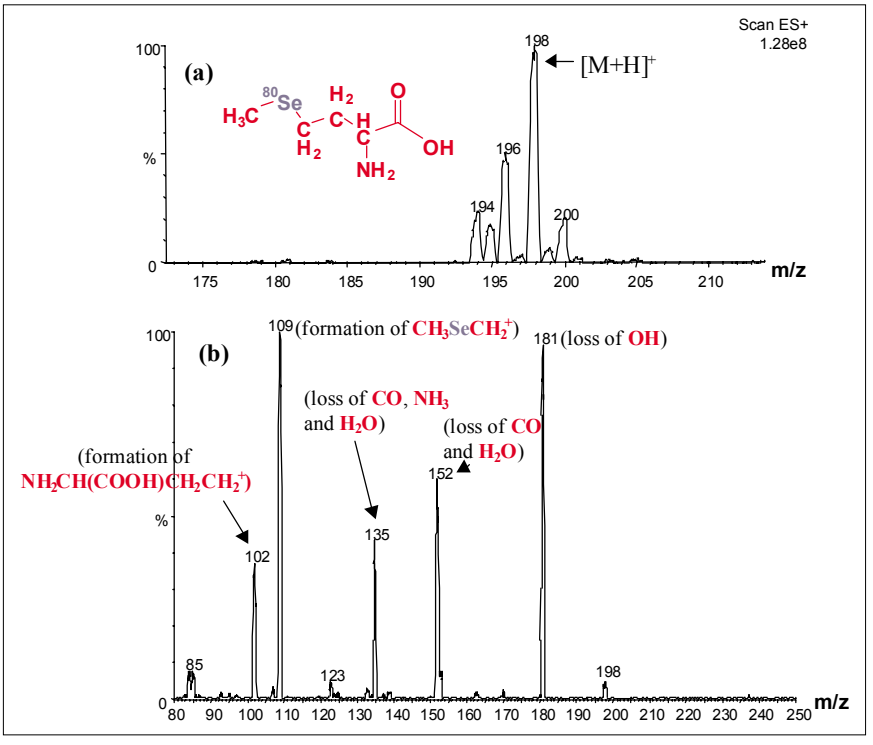

Figure 4. (a) Observed ESI-MS isotopic pattern and (b) ESI-MS/MS product ion spectrum of the precursor ion *m/z* 198 for the selenomethionine peak in a yeast enzymatic hydrolysate (adapted from Reference 19).

Future analytical developments

More extensive selenium speciation studies in high-Se food are required to fully establish the links between health benefits and particular selenium species but such studies are dependent on further improvements to the analytical methods. Extension of the speciation work to the analysis of clinical samples (e.g. human serum) will be essential to perform studies on Se bioavailability. The production of isotopically-enriched Se-compounds will also help in the characterisation of candidate "speciated" reference materials via isotope dilution techniques and in the validation of new speciation methodologies.

There is a need to develop chromatographic separation methods that can be sequentially coupled on-line to both inorganic and organic mass spectrometry. The identification of target species at low concentration levels is needed in a wide range of complex matrices, including clinical samples, in order to advance research into the efficacy of supplements. This will require the development of new

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and more efficient sample clean-up and preconcentration methods. Identification of previously unknown Se-compounds, for which authentic standards are not available, demands the combined use of a range of analytical techniques (e.g. MS, NMR) to elucidate their structural characteristics. Confirmation of Se species revealed by means of chromatography and element-specific detection may involve the accurate and precise molecular mass measurement of the unknown isolated species and of the product ions obtained from the precursor ion after collision-induced dissociation (tandem MS). This could be achieved by using, for example, time-of-flight (ToF) or Fourier transform (FT) mass spectrometry.

Conclusions

The identification and measurement of selenium compounds is essential for medical and nutritional research into the use of selenium-containing drugs or supplements, in the development of safe and effective products, in the understanding of their chemopreventive activity, and for future regulation of their production and use. At present most analytical methods available to undertake these measurements cannot be properly validated; there is a clear need for new calibration standards, isotopically-enriched species and reference materials, which will be useful to ensure that data from newly developed methods are traceable and valid. However, recent developments based on novel extraction methods and advanced mass spectrometry offer the prospect of rapid advances in our capability to identify and measure key selenium species at low concentrations in both food and clinical samples. LGC and other institutes around the world are also collaborating in the development of appropriate standards and reference materials which will ensure that the new methodology can be applied reliably by laboratories in the clinical, pharmaceutical, food and academic sectors.

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