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Chiral speciation of trace elements: approaches to the speciation of selenoaminoacid enantiomers in biological samples†

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The "state of the art" of chiral trace element speciation, that is, the recognition of the particular enantiomer in which the trace element sought is occurring, is given here. The importance of such studies and speciation information is highlighted. Recent advances and trends in the development and applications of analytical approaches for chiral speciation of trace elements in biological material are also reviewed. A comparative overview is given of existing selenoaminoacids chiral speciation methods, based on hybridization of an adequate chiral separation technique (*e.g.*, high-performance liquid chromatography, gas chromatography or capillary electrophoresis) coupled on-line with selenium specific detection by inductively coupled plasma mass spectrometry (ICP-MS). Applications of these hybrid methodologies, reported so far in the literature, to selenoaminoacids chiral speciation in biological real samples are also reviewed.

1. Introduction

Any modern chemist is well aware of the existence of a very important type of space isomerism in some organic compounds having asymmetric centers, axes, or planes in their molecule. The two spatial isomers resulting from one "chiral" center in a molecule are called enantiomers. Of course, they are not identical, but one of them is the mirror image of the other.

Since Pasteur's very first resolution of a mixture of such two isomers (racemate) of sodium and ammonium tartrate crystals, carried out manually back in 1848, we have witnessed a huge accumulation of such stereochemical knowledge. This is particularly so since the 1980s.[1] At the beginning of the third millennium, nature's amazing ability to produce and convert chiral compounds with remarkable stereospecificity is both fascinating and extremely well documented. Particularly in biological organisms, the interaction between biologically active compounds and receptors (*e.g.*, proteins, enzymes) is most often characterized by a high, or even complete, enantiomeric specificity. At lot of evidence has been accumulated so far showing that great differences can be observed related to biological activity, potency, toxicity, transport and routes of metabolism of individual enantiomers. This fundamental and academic interest suddenly became a practical issue after recognition in 1979 that the dramatic effects of thalidomide in pregnant women, observed in the sixties, were related to chirality; in fact, (*R*)-thalidomide has the desired antinause effect, while the (*S*) enantiomer is teratogenic. Thus, the deformed babies born after their mothers had taken thalidomide could have been a consequence of the production and eventual ingestion of the racemate drug. It is not surprising that chiral recognition has become today a most important topic in the pharmaceutical industry (*e.g.*, out of the 200 most popular drugs it seems that around 60% of them are "chiral"). Knowing accurately the stereochemical composition of organics used as pharmaceuticals is now compulsory by law and is extending to herbicides, pesticides, antibiotics, *etc.*[2–7]

Also, speciation of trace elements is arousing a great interest in modern analytical chemistry.[8] Moreover, trace element speciation in the particular field of biological systems is proving to be a great challenge, as many metal–biomolecules formed in living organisms are still unknown.[9] From the above narrative on chirality of compounds it seems clear that a given trace element could be present in different enantiomeric forms or species. Thus, for a complete speciation of such a trace element, the recognition of the particular enantiomer in which the element is occurring (so-called "chiral speciation") should be possible. Therefore, chiral speciation analytical approaches for trace elements, particularly in biological materials, are needed nowadays.

A number of approaches[1,10–14] have been used for the chiral recognition of organic compounds, including polarimetry, circular dichroism, nuclear magnetic resonance, chromatography with conventional detectors such as UV/VIS, or capillary electrophoresis with UV/VIS detection. Only recently[15] some attention has been paid to the use of mass spectrometry (MS), alone or in combination with chromatography. It is well established by now that hybrid techniques are preferred for trace element speciation. In fact, chromatographic techniques coupled to atomic detectors seem to offer the most practical and powerful approach.[9] Not surprisingly, the first papers on this topic of "chiral speciation" used hybrid high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS)[16–19] or gas chromatography inductively coupled plasma mass spectrometry (GC-ICP-MS)[20] for the recognition and determination of enantiomeric forms in selenoaminoacids. However, the high separation efficiency of capillary electrophoresis (CE) and its present wide applicability to various classes of compounds make this latter separation technique particularly amenable to separating chiral molecules.[13] Unfortunately the on-line coupling of CE to MS and ICP-MS detectors is not so straightforward. Therefore, in spite of the continuous interest in CE-MS and CE-ICP-MS couplings for elemental speciation studies in biological samples,[21] no applications of such techniques to chiral speciation analysis have appeared so far.

In this paper the "state of the art" of chiral speciation, virtually restricted so far to Se-aminoacids speciation, is given and possible new ways and approaches to expand the scope of the field are discussed.

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2. The tools of the trade

A successful approach to chiral speciation analysis of trace elements in biological samples (biological fluids, food, nutritional supplements, *etc.*) requires first the use of very selective (enantioselective) methodologies, able to discriminate between the two enantiomeric forms of the chiral compound of interest present in a complex matrix sample. Secondly, the method has to be very sensitive because the chiral compound is usually a trace constituent in the sample and is often not present as the racemate (*e.g.*, in many cases one of the enantiomers is an impurity of the other). So, to carry out enantioselective trace element speciation analysis, we should rely on analytical separation systems with a high separation power allowing the accurate determination of impurities amounting to less than 1% of the enantiomer, which is a widely accepted criterion of purity for enantiomeric compounds. This could be greatly facilitated by enantioselective separation systems that simultaneously provide high efficiency/resolution capability for enantiomer separation and allow the on-line coupling of highly sensitive and element-specific detection methods like ICP-MS. In this way, the coupling of a chiral separation technique such as HPLC, GC (or CE) with an ICP-MS detector appears to meet the requirements of enantioselectivity and sensitivity.

2.1. Enantiomer separation strategies

Two major strategies for enantiomer separation are available based on formation of diastereoisomers with the enantiomers of the analyte. The indirect approach consists of reacting the enantiomers with an optically pure chiral compound. After the reaction, the diastereoisomers of the derivatized analyte can be separated using conventional (achiral) separation methods, which is a major advantage of this approach.

Direct enantiomer separation is based on the reversible formation of diastereoisomeric complexes between enantiomers of the analyte and a chiral selector. This ''selector'' is generally bound to a chromatographic support material [chiral stationary phases (CSP)]. The difference in stability between the two diastereomeric complexes leads to a difference in retention time and hence separation.

Within the diastereoisomeric analyte–chiral selector complex, chiral recognition is based on the three-point rule, proposed by Dalgliesh.[22] The direct method is presently the most widely used technique for enantiomeric separations, particularly HPLC-CSP with more than two hundreds of commercially available CSP.[23]

Although a broad spectrum of chiral selectors, with quite different chemical structures, have been investigated in order to generate enantioselectivity in HPLC,[1,12,14] GC[1,10] and CE,[13] most separations are based today on the use of the macrocyclic compounds: cyclodextrins, crown ethers and antibiotics.[24]

Cyclodextrins (CD) are natural cyclic oligosaccharides, which have a stereospecific toroidal (doughnut-shaped) structure. Individual enantiomer molecules can get into the cavity of the CD to form inclusion complexes and, having different binding constants, it is possible to resolve them.[25–27] The native CD, α, β and γ, and several derivatized CD have been successfully used as CSP (''Cyclobond'' columns) in reversed-phase HPLC for the resolution of a great variety of enantiomeric compounds including drug substances and derivatized aminoacids.[1,14,28]

Fused-silica capillary columns coated with alkylated CDs have also been developed for enantiomer separations by GC.[10] CDs have been used as well as buffer additives for chiral separations by CE.[13,29] Moreover chiral resolution by CD-mediated micellar electrokinetic chromatography (CD-MEKC) has been reported.[13,29,30]

The second type of chiral selector is crown ethers, synthetic macrocyclic polyethers. Chiral crown ethers, able to form stereoselective inclusion complexes with ammonium cations, have been immobilized on chromatographic supports, yielding commercial CSP (''Crownpack CR'' columns) for the HPLC separation of enantiomers containing a primary amine functional group at or near the stereogenic center.[31–33] The mobile phases used on this CSP are limited to (10^{-3}–10^{-2} M) perchloric acid.[32,33] This principle of chiral separation has also been introduced to CE for the chiral resolution of underivatized amino acids[34,35] and other compounds containing primary amino groups.[33,36]

Finally, macrocyclic glycopeptide antibiotics, such as vancomycin and teicoplanin, are the newest class of chiral selectors in HPLC (''Chirobiotic'' columns).[37–39] They have multiple chiral centers and several functional groups (amine, carboxylic acid, hydroxyl, aromatic, hydrophobic pockets) that are capable of multiple interactions to enable chiral recognition. The primary interactions for enantio-recognition are assumed to be ionic interactions.[37–39] Armstrong and coworkers[40–43] for CE enantioseparation of several anionic compounds have also introduced these macrocyclic glycopeptides antibiotics.

2.2. ICP-MS as on-line detection system in HPLC, GC and CE

ICP-MS provides a highly effective element-specific detection system for chiral speciation studies. Coupled on-line after an appropriate separation technique (*e.g.*, HPLC, GC and CE), ICP-MS offers excellent sensitivity and selectivity for multi-element identification and quantitation of inorganic and organometallic compounds with the additional capability of isotopic analysis.[44] Nevertheless, these couplings of instruments, which were never designed to be coupled, still suffer from a number of drawbacks (mainly inherent to the separation/detection interface). This is especially true for GC-ICP-MS and CE-ICP-MS, whereas HPLC-ICP-MS interfacing is more straightforward.

Typical mobile phase flow rates in HPLC are of the order of 0.5–2 mL min^{-1} and so fit well with the nebulization sample uptake rate of most commercial concentric or direct nebulizers of ICP-MS. Thus, coupling HPLC to ICP-MS is rather easily accomplished by passing the column effluent through the shortest possible narrow bore tube, made of an inert material, up to the pneumatic nebulizer. Of course a serious limitation of this conventional nebulization is the low analyte transport efficiency (1–3%) to the plasma. Therefore, many variations of the conventional pneumatic nebuliser/spray chamber sample introduction system have been described in the literature to improve sensitivity of HPLC-ICP-MS couplings even further.[45,46]

Another problem encountered with direct HPLC-ICP-MS interfacing arises when using mobile phases containing high concentrations of salts, or buffers or high percentages of organic solvents. The salt content of the mobile phase can cause rapid erosion and clogging of the nebulizer and of the sampling- and skimmer cones. Organic solvents will cause plasma instability and deposition of carbon on the sampling cone and torch. Thus, a compromise in the chromatographic conditions is often made by using a lower salt content ($<2\%$ total dissolved solid) and lower organic solvent contents in order to obtain an efficient separation while maintaining adequate ICP-MS detector performance.[45,46]

As GC column effluents are in the gas phase, coupling GC with ICP-MS provides nearly 100% analyte transport efficiency, and this results in comparatively low detection limits.[47] The analyte is also more efficiently ionized in the plasma, as it is already in the vapor form, without requiring additional plasma energy.[47] However, GC-ICP-MS interfacing is somewhat more complicated than HPLC-ICP-MS. Although several approaches have been described in the literature,[48–50] the

most frequently used configuration for capillary GC-ICP-MS interfacing involves connection of the capillary column to the inner tube of the torch using a transfer line between the GC oven and the plasma torch. The transfer line usually consists of a tube of an adequate material into which the analytical column is passed. Typically, but not always,[50] the transfer line is heated in order to prevent analyte condensation. Because the carrier gas flow rate when using capillary GC columns is very low, it is necessary to introduce a make up gas in order to open the central channel in the plasma and help to carry the analyte from the GC column into the plasma. This make up gas flow must be optimized to minimize peak broadening and maximize analyte transport.

The coupling of CE to ICP-MS has started to receive attention in recent years; the first paper describing the technique was written in 1995 by Olesik *et al.*[51] Since then, about 30 reports concerning coupling of CE to ICP-MS have appeared in the literature.[52] This is probably due to the complexity of the coupling: no conventional nebulizers available seem to work adequately at the very low flows used in CE. Moreover, the small sample volume (less than 100 nl) appropriate for CE requires that the transport efficiency of the analytes into the ICP should be as high as possible. Therefore, at present the challenge of CE-ICP-MS coupling is to achieve an effective introduction of microflows from the capillary into the ICP without excessive band broadening. In addition, the CE-ICP-MS interface should provide an electrical connection to the outlet end of the capillary, while preventing the introduction of a laminar flow in the capillary (*e.g.*, as caused by the suction effect when a pneumatic nebulizer is used in the interface[51]). All these considerations have been most commonly addressed by using a conductive sheath flow (make-up) electrolyte around the end of the capillary in combination with different commercial nebulizers such as pneumatic,[51,53–57] direct injection,[58] ultrasonic[59,60] and micro-concentric.[61,62] Also, an interface using on-line volatile species generation is possible.[63,64]

3. Chiral speciation of selenoaminoacids

Selenium is an essential micronutrient for most living organisms including humans. It is a component of procaryotic and eukaryotic antioxidant proteins, which have roles in antioxidant defenses, thyroid function, reproduction capacity and protection against tumors and virus infections.[65] Thus, it is not surprising that Se-supplements have become today very popular in the diet of man and animals.

The effectiveness of this dietary selenium supplementation is, however, dependent not only on the total trace element concentration,[66] but also on the particular species of selenium consumed.[67,68] Selenoaminoacids are the principal dietary form of selenium (selenomethionine is derived from plants and selenocysteine from animals) as these Se-forms are adsorbed to a greater extent than inorganic species, are less toxic than inorganic selenium species (selenate and selenite) and can be used for the biosynthesis of various selenoproteins (containing covalent C–Se bonds) in a more straightforward way.

Moreover, aminoacids are probably the most important class of "chiral" compounds[24] and different bioavailability, biochemical behavior and roles can be expected for the different possible enantiomeric forms of a given selenoaminoacid. Thus, research work on the biological activity and stereochemical pathways of selenoaminacids in animals and man requires access to adequate analytical techniques for "chiral speciation" of selenoaminoacids enantiomers in real samples at the low concentration levels of selenium existing naturally.

In spite of this importance, very little attention has been paid so far to this problem. In fact, only five papers on this topic have appeared in the literature,[16–20] all of them based on hybridization of a chiral chromatography separation with selenium-specific detection by ICP-MS.

3.1. Chiral speciation of selenoaminoacids by HPLC-ICP-MS

The separation of selenomethionine diastereoisomers by conventional reversed-phase HPLC after derivatization with a chiral reagent has been reported by Hansen and Poulsen[69] with UV absorbance detection. The first chiral HPLC-ICP-MS hybrid methodology for the chiral speciation of selenomethionine enantiomers was developed in our group three years ago.[16] A β-CD column (Cyclobond I) for the HPLC chiral separation was used. Unfortunately the elevated methanol content of the mobile phase, necessary for the enantiomeric separation,[16] produced high plasma instability and even eventual extinction of the plasma. Therefore, the first direct coupling of the developed chiral selenoaminoacid separation on a β-CD column to an ICP-MS was unsuccessful in practice. This problem was overcome by resorting to a previously developed hydride generation (HG) interface.[70] Unfortunately the attained detection limit was very poor (only 70 μg L^{-1} as Se for each enantiomer). Moreover, the method required pre-column derivatization of the selenoaminoacids in order to achieve chiral recognition on the β-CD column. It is clear that alternative approaches avoiding precolumn derivatization should be preferable.

In this vein, Sutton *et al.*[17] recently described the enantiomeric separation of underivatized selenoaminoacids by HPLC-ICP-MS using a chiral crown ether column. As shown in Fig. 1 (ref. 17) this chiral crown ether-based column enabled almost baseline resolution of all the enantiomers investigated in 60 min. However, the separation was found to be very sensitive to changes in temperature (a fluctuation of ±1 °C resulted in a decrease in resolution) and the reported sensitivity was rather poor for real sample enantiomeric determinations with detection limits of 15.5 μg L^{-1} (6.2 μg L^{-1} as Se) and 49.5 μg L^{-1} (19.9 μg L^{-1}as Se) for L-and D-selenomethionine, respectively,[17] with an injection volume of 20 μL. This chiral HPLC-ICP-MS method was further investigated by Ponce de Leon *et al.*[19] and applied to the enantiomeric separation of several selenoaminoacids, including Se-lanthionine, Se-methylselenocysteine, Se-cystine, Se-cystathionine, Se-adenosylselenohomocystine, γ-glutamyl Se-methylselenocysteine, Se-ethionine and Se-homocystine. Although this chiral crown ether column enabled the individual enantiomeric separation of the

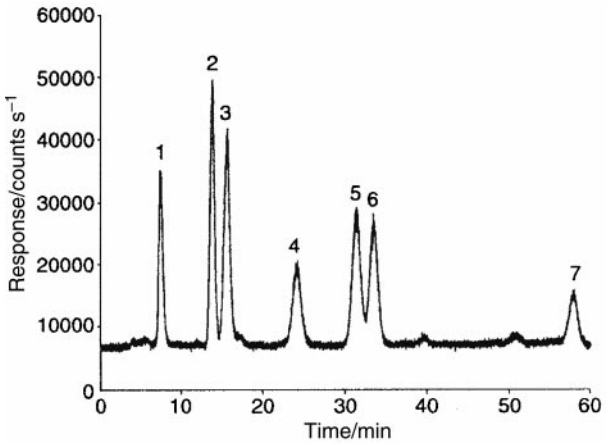

Fig. 1 HPLC (Crownpack CR(+) column)-ICP-MS chromatogram of a racemic mixture of selenoaminoacids. Mobile phase: 0.1 M $HClO_4$ (pH 1), 0.5 ml min^{-1} for 35 min then increased to 1.0 ml min^{-1}. Peaks: 1 = L-selenocystine; 2 = L-selenomethionine; 3 = *meso*-selenocystine; 4 = D-selenocystine; 5 = D-selenomethionine; 6 = L-selenoethionine and 7 = D-selenoethionine. Reprinted with permission from ref. 17.

selenoaminoacids investigated, their simultaneous separation (24 potential enantiomeric peaks in a mixture) was not achieved.[19]

Recently, a teicoplanin–bonded chiral stationary phase (Chirobiotic T) introduced by Armstrong and co-workers[39] has been shown to be able to resolve a variety of underivatized aminoacids using hydro-organic mobile phases containing very low percentages of organic modifier and no buffer or salts of any kind.[39] These features offer a very convenient approach to the direct coupling of such a column with the nebulizer of the ICP-MS detector. Fig. 2 shows the chromatogram obtained in our research group[18] for a racemic mixture of underivatized selenomethionine [Fig. 2(a)] and selenoethionine [Fig. 2(b)] when the teicoplanin column was coupled with selenium specific ICP-MS detection.

The sensitivity of this HPLC (teicoplanin column)-ICP-MS methodology was found to be very good, with detection limits for selenomethionine of 1.9 μg L^{-1} (0.8 μg L^{-1} as Se) for each enantiomer, for an injection volume of 20 μL.[18] This means a detectability for Se chiral speciation of around 60–200 times better than that reported by Sutton *et al.*[17] using HPLC-ICP-MS with a crown ether chiral column.

3.2. Chiral speciation of selenoaminoacids by GC-ICP-MS

The most frequently used chiral selectors in capillary GC are CDs derivatives. However, some other compounds, such as chiral amino acid derivatives and chiral metal coordination compounds, have also been found to be useful as chiral selectors.[10] Particularly fused silica capillary columns coated with L-valine-*tert*-butylamide (Chirasil-L-Val) have been shown to be most suitable for the optical resolution of *N*-trifluoroacetyl (TFA)-*O*-alkyl derivatives of several amino acid enantiomers *via* multiple hydrogen bond interactions.[10,71]

The potential of coupling chiral GC, on the Chirasil-L-Val column, with selenium-specific detection by ICP-MS for the separation and determination of DL-selenomethionine enantiomers has been recently demonstrated in our research group.[20] The GC column was coupled to the ICP-MS through an interface developed in our laboratory.[50] A typical chromatogram of a racemic mixture of DL-selenomethionine (derivatized to form *N*-TFA-*O*-isopropyl-derivatives) obtained by the GC-ICP-MS method is shown in Fig. 3 (ref. 20).

The reported hybrid chiral GC-ICP-MS method proved to be very sensitive, as the detection limits were found to be 0.25 μg L^{-1} of selenomethionine (0.1 μg L^{-1} as Se) for each enantiomer,[20] with an injection volume of 1 μL. Thus, this hybrid technique offers the more sensitive chiral speciation of Se-aminoacids so far.

3.3. Chiral speciation of selenoaminoacids by CE-ICP-MS

The use of CE to obtain chiral separations of aminoacids has been already reviewed.[72] CE has also been reported to separate the enantiomers of some selenoaminoacids by the addition of vancomycin[73–75] or sulfated β-CD[75] as chiral selectors to the running electrophoretic buffer. UV absorbance detection was used in such studies and required the derivatization of the selenoaminoacids with a quinoline derivative.

Separation of naphthalene-2,3-dicarboxaldehyde (NDA)-D,L-selenomethionine and selenoethionine derivatives using a CD-micellar electrokinetic chromatography (MEKC) methodology has been investigated in our laboratory. Experiments carried out with UV detection at 230 nm showed that an electrophoretic buffer containing a mixed micellar system of achiral SDS and chiral TDC plus β-CD provided a good resolution ($R = 1.25$) of the selenomethionine enantiomers [Fig. 4(a)].[76] Unfortunately, the on-line coupling of this chiral separation by CD-MEKC with selenium specific ICP-MS detection using a conventional Meinhard nebulizer for interfacing CE with ICP-MS was unsuccessful; the optical resolution obtained between the enantiomers was lost, as shown in Fig. 4(b),[76] probably due to the laminar flow induced by the suction effect of the Meinhard nebulizer and its dead volume. Coupling of this chiral CD-MEKC method to ICP-MS detection *via* a high-efficiency nebulizer (HEN) in

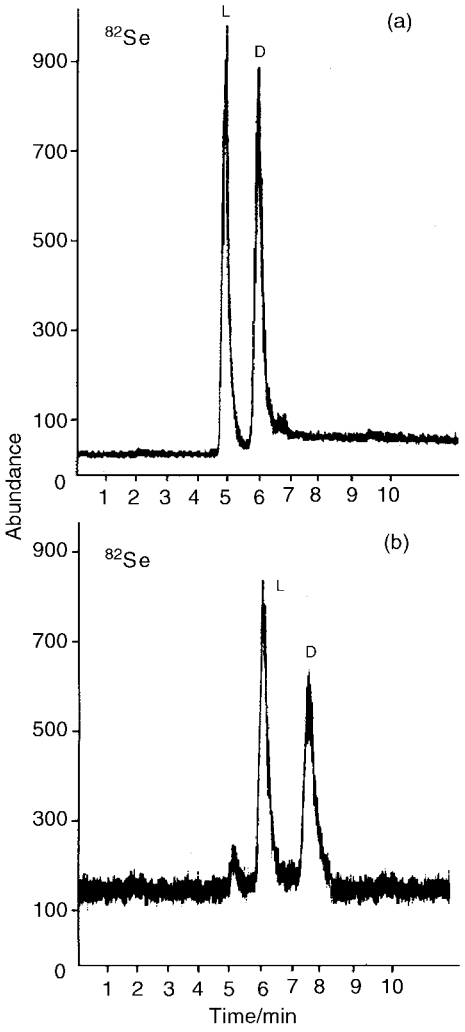

Fig. 2 HPLC (Chirobiotic T column)-ICP-MS chromatogram of a racemic mixture of: (a) selenomethionine and (b) selenoethionine. Mobile phase: 2% v/v methanol–water, 1.0 ml min^{-1}. Reprinted with permission from ref. 18.

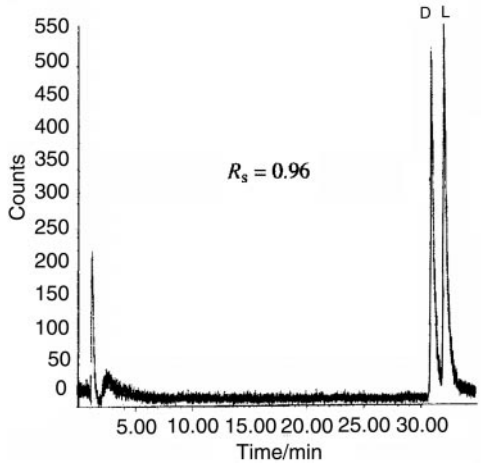

Fig. 3 GC (Chirasil-L-Val column)-ICP-MS chromatogram of a racemic mixture of selenomethionine (as *N*-TFA-*O*-isopropyl derivatives). Reprinted with permission from ref. 20.

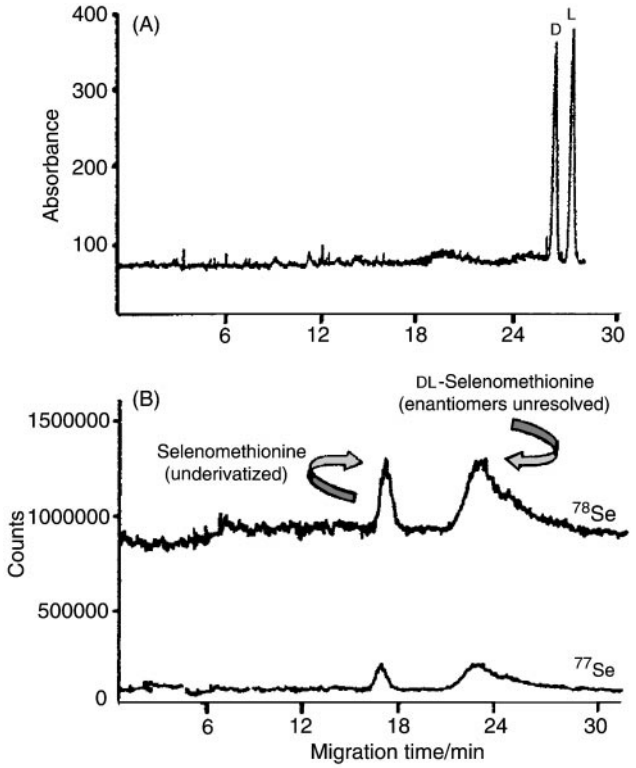

Fig. 4 CD-MEKC electropherogram of a racemic mixture of NDA-selenomethionine derivatives with: (a) absorptiometric detection (230 nm) and (b) ICP-MS detection. Reprinted with permission from ref. 76.

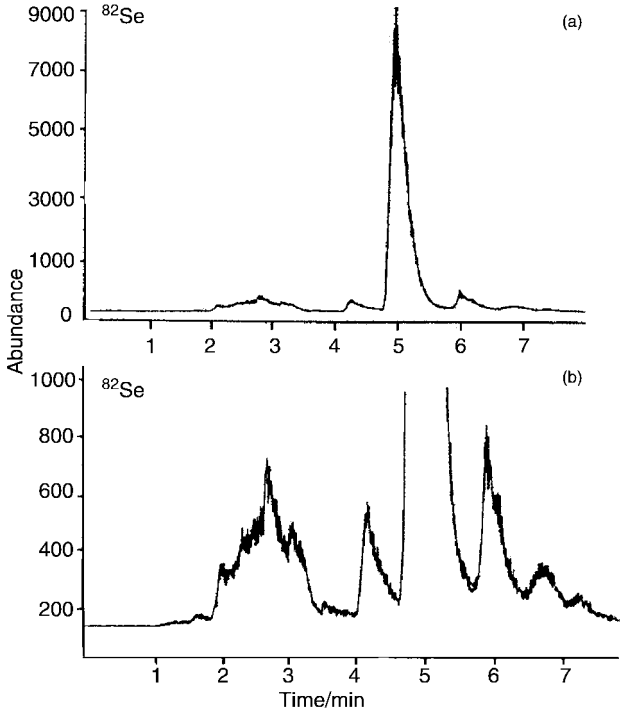

Fig. 5 HPLC (Chirobiotic T column)-ICP-MS chromatogram of a "selenized yeast" after (a) enzymatic hydrolysis with protease and (b) same as in Fig. 5(a) but with an expanded *y*-axis scale. Reprinted with permission from ref. 18.

order to avoid the suction effect is now under investigation in our laboratory.

4. Chiral speciation of selenium in real samples

As mentioned earlier, selenomethionine has been reported to be more bioavailable and less toxic than inorganic selenium species.[77] Thus, free selenomethionine and selenized yeast, which contain peptidic selenomethionine as the major selenium species,[78] are now being used extensively as a convenient source of selenium for human dietary supplementation. Therefore, application of the previously developed chiral HPLC-ICP-MS and GC-ICP-MS methodologies for the enantiomeric resolution and determination of selenoaminoacid enantiomers was focused on the direct chiral speciation of selenomethionine in such nutritional real samples.[17–20]

The problem of chiral speciation of selenomethionine in a selenized yeast sample was first looked at three years ago.[16] Chiral GC-ICP-MS for analysis and enzymatic hydrolysis extraction with protease for sample preparation eventually gave a satisfactory GC-ICP-MS chromatogram of the yeast sample hydrolysate.[20] In that chromatogram a selenium peak eluting at the retention time of the L-selenomethionine enantiomer was obtained. Interestingly, another small selenium peak corresponding to the D-selenomethionine enantiomer was also observed.[20] The GC-ICP-MS chiral analysis of a parenteral solution sample for human use was also reported in the same paper. Results obtained indicated that selenomethione in the analyzed sample (diluted 1 : 40 with ultrapure water) was present as a racemate (information which was not given on the commercial label).[20]

Further work in our research group[18] for chiral analysis of the selenium-enriched yeast was carried out using chiral HPLC (teicoplanin column)-ICP-MS, after both water extraction and enzymatic hydrolysis of the yeast. A typical chromatogram of the enzymatic extract, showing the presence of D- and L-selenomethionine, together with other unidentified selenium species, is given in Fig. 5 (ref. 18). The content of D- and L-selenomethionine in the enzymatic extract, which is about 30 times higher than that observed in the water extract, was determined by standard additions-chiral HPLC (teicoplanin column)-ICP-MS and it was found to be 5 ± 1 mg L^{-1} and 23.9 ± 7 mg L^{-1} (as Se), respectively. This value amounted to around 11% for the D- and 51.5% for the L-enantiomer of the total selenium present in the enzymatic extract, found to be 47 ± 1 mg L^{-1} by direct nebulization in the ICP-MS.[18]

Five commercial selenium yeast nutritional supplements were analyzed by Sutton *et al.*[17] using chiral HPLC (crown ether column)-ICP-MS, after pepsin enzymatic hydrolysis. In two of the yeast samples analyzed selenomethionine was not detected and only one sample was found to contain D- and L-selenomethionine.[17] However, it is necessary to stress that for all the yeast samples the concentration of selenium, determined using the chromatographic method, was significantly lower than the total selenium concentration, determined by analyzing the digest samples (nitric acid and simulated gastric digestion) directly by ICP-MS.[17] In other words, pepsin enzymatic hydrolysis was not able to cleave all the peptide bonds in the yeast sample. Therefore some of the selenoaminoacids present could remain as protein-bound (and so not available for the proposed separation).

Other commercially available nutritional supplements, two multivitamin supplements containing selenium as selenate, a supplement containing selenium as selenite and two yeast free supplements containing selenium as L-selenomethionine, were also analyzed by chiral HPLC (crown ether column)-ICP-MS and the selenium species that were unretained in the chiral column were successfully identified by anion-exchange chromatography.[17]

In a further work[19] the same group applied this crown ether column-ICP-MS method to identifying the selenium species present in selenium enriched onion, garlic and yeast. Two different extraction methods for the samples were compared: water extraction and a pepsin enzymatic extraction. Though

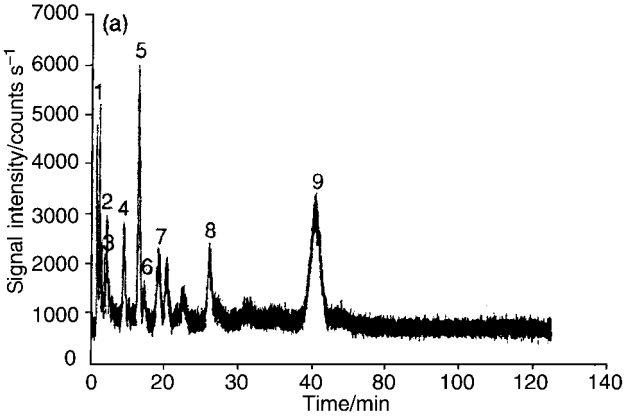

Fig. 6 HPLC (Crownpack CR(+) column)-ICP-MS chromatogram of 1922 Se Nutrition 21 yeast sample after water extraction. Peaks: 1 = Se-lanthionine; 2 = D-Se-methylselenocysteine; 3 = L-Se-methylselenocysteine; 4 = L-Se-cystathionine; 5 = L-selenomethionine; 6 = Se-adenosylselenohomocystine or Se-homocystine; 7 = γ-glutamyl Se-methylselenocysteine; 8 = Se-adenosylselenohomocystine; 9 = unknown. Reprinted with permission from ref. 19.

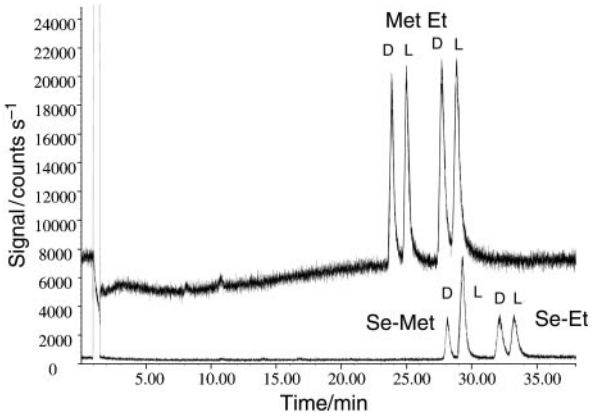

Fig. 7 GC (Chirasil-L-Val column)-ICP-MS(DF) chromatogram of a racemic mixture of selenomethionine and methionine (as *N*-TFA-*O*-isopropyl derivatives). Experimental conditions as in Fig. 3.

virtually the same species were observed for both extracts, the extraction efficiency, especially for selenomethionine, was several times lower using water extraction. The major component in the enriched yeast investigated was L-selenomethionine while D-selenomethionine was not detected.[19] The many species identified are given in Fig. 6.

A commercially pure L-selenomethionine sample was also analyzed in our research group using both chiral HPLC (β-CD column)-HG-MW-ICP-MS[16] and chiral GC-ICP-MS methodologies.[20] The results of these analysis agreed well and showed the presence of an impurity of the D-enantiomer in the sample.[16,20] The relative level of D-selenomethionine in such as commercial sample was found to be of about 8% of the total DL-selenomethionine content.[20]

5. Final remarks and future directions

It appears from the above narrative that chiral speciation of trace elements in biological material is today a fascinating new frontier in analytical chemistry and represents a real analytical challenge.

There is an urgent need to develop hybrid robust analytical methodologies able to tackle trace element chiral speciation in real samples. Only with such adequate tools will chiral or stereochemical composition of species of essential, toxic and therapeutic trace elements in biology and medicine be clarified. Thus, new studies of the role of trace elements and their compounds (particular species) in living organisms can be undertaken, particularly on interactions between receptors (*e.g.*, proteins) and chiral trace element species to ascertain the existence (or otherwise) of enantiomeric specificity.

In this vein, it seems that chromatography-ICP-MS is the hybrid technique of choice so far for chiral speciation analysis in real samples. GC-ICP-MS offers the highest sensitivity, while HPLC-ICP-MS seems to afford the most robust approach for real samples. Although attractive, speciation of too many compounds in a single run with a chiral column (Fig. 6) may become misleading for very complex matrices of real life analysis. Thus, in some cases, switched column technologies are advisable (that is, a non-chiral separation column switched adequately to a chiral one for chiral speciation). Moreover, the ICP-MS multielemental capabilities should be also used in trace element speciation in biological material research.[79] In fact, multielemental chiral speciation in a single injection has already been demonstrated in our laboratory following Se and S *via* an HPLC-double focusing (DF)ICP-MS experiment, as shown in Fig. 7. Of course, there are many interelemental interactions in living organisms (*e.g.*, positive effect of selenium on As and Hg intoxication), which could be elegantly studied with such powerful tools.

CE-ICP-MS holds great potential for chiral speciation, but realization of such potential is still to come, as for simultaneous multielemental chiral speciation mentioned above.

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