

Development of chiral HPLC for selenoamino acids with ICP-MS detection: application to selenium nutritional supplements

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The enantiomeric separation of three underivatized selenoamino acids, D,L-selenocystine, D,L-selenomethionine and D,L-selenoethionine, with UV and ICP-MS detection is described. An HPLC column with a chiral crown ether stationary phase and a mobile phase of 0.10 M HClO₄ was used. Absolute detection limits obtained with UV detection ranged from 34.5 to 47.1 ng whereas those obtained with the plasma detector were *ca.* 40–400 times better. The separations with either detector were good, with little detector effect on the resolution. Ten commercially available dietary selenium supplements were analyzed using the chiral column to identify and quantify the selenium species present with both detection modes. Selenium species were easily identified using ICP-MS detection, whereas UV detection was not viable because of interferences from the sample matrix and inadequate sensitivity. Selenium species that were unretained using the chiral column were identified using anion exchange chromatography. Total amounts of selenium in the samples were also measured using a conventional digestion and an enzymatic digestion with ICP-MS detection.

Introduction

The necessity for selenium in the human diet is well established and daily amounts of 0.8–1.7 $\mu\text{mol L}^{-1}$ are required to prevent selenium deficiency.¹ Studies in the past 2 years on the administration of selenium supplements have shown that selenium may inhibit oxidative damage and thus reduce the incidence of some types of cancer.² There is, however, only a narrow range between the daily amount of selenium that may be beneficial or toxic to humans³ and such effects are dependent on the chemical form of the selenium species.⁴ Since there are many forms of selenium,^{5,6} which may be present in only very small amounts, selenium speciation methods are required with high selectivity and very low detection levels.

Selenium supplements are often marketed as the selenomethionine form. This type of α -amino acid is chiral because of the asymmetric α -carbon atom. It is therefore useful to develop chiral separations for these substances that will allow the highest specificity possible at physiological concentration levels. The combination of a highly selective chiral separation, such as that selective for primary amines, with a method of detection viable to ultra-trace levels, such as inductively coupled plasma mass spectrometry (ICP-MS), is therefore highly desirable.

Speciation of inorganic selenium and organoselenium compounds using non-chiral HPLC with ICP-MS detection methods have been described previously.^{5,7–22} Anion exchange chromatography,^{8–12,16,21} reversed phase chromatography,^{7,15} ion pair chromatography^{14,17–20} and size exclusion chromatography,¹³ coupled to ICP-MS, have all been used; however, no information concerning the enantiomeric distribution of the chiral compounds is obtained when using these techniques.

The use of capillary electrophoresis (CE) to obtain chiral separations of amino acids has been reviewed.²³ This separation technique has been used to separate the enantiomers of selenoamino acids by the addition of vancomycin^{24–26} or sulfated β -cyclodextrin²⁶ as chiral additives to the electro-

phoretic buffer. UV absorbance detection was used in these studies and required the derivatization of the selenoamino acids with a quinoline derivative to permit detection. UV absorbance detection, without sample preconcentration, was not sensitive enough to permit the detection of the low levels of selenoamino acids present in complex samples, such as selenium supplements, and attempts at interfacing ICP-MS to obtain better detection capabilities have not been successful to date. Hence chiral LC coupled with ICP-MS detection may provide a better option.

The use of a β -cyclodextrin column for the separation of the enantiomers of selenomethionine has been described.²⁷ In this study, the amino acid was derivatized using *o*-phthalaldehyde or naphthalene-2,3-dicarboxaldehyde to allow conventional fluorimetric detection. Such a derivatization step, however, is undesirable because it lengthens the sample preparation time, and requires additional validation because it may be a potential source of contamination, may induce racemization or may complicate the separation.

An alternative method for the separation of selenoamino acids by HPLC without derivatization is therefore desirable. Several chiral stationary phases are available and have been the subject of a recent review.²⁸ These include chiral crown ether phases. Chiral separations using such columns arise owing to the formation of transient diastereomeric complexes between the enantiomers and the chiral ligand of the stationary phase. Derivatization is not always necessary, depending on the specific analyte, type of column and method of detection used.²⁸

In this work, a commercially available HPLC column with a chiral crown ether stationary phase was used to perform enantiomeric separations of selenoamino acids without derivatization. To our knowledge, this is the first time that such a separation has been performed using non-derivatized selenoamino acids. This type of column is highly effective for separating compounds with primary amino groups under acidic conditions. It has been used to achieve enantiomeric separations

of amino acids,^{29–32} dipeptides³³ and amines.³⁴ The recommended mobile phase for such a column is dilute perchloric acid (pH 1). Fortunately, this mobile phase is well suited for ICP-MS detection as no organic mobile phase constituents are present.³⁵ Canals *et al.*³⁶ showed that perchloric acid behaves similarly to nitric acid in terms of sample uptake rate, primary and tertiary drop size distributions, total analyte transport rate and plasma temperature at equivalent concentrations.

In this study, the use of a chiral crown ether column was investigated for the identification and quantification of various selenoamino acids. Both UV detection and ICP-MS detection were used in tandem. This study was applied to complex samples by investigating over-the-counter nutritional supplements. The samples were then analyzed to compare UV with ICP-MS detection, to determine the selenium species and to identify and quantify the enantiomers present. Anion exchange chromatography was used to supplement the chiral chromatography and finally speciation information was compared with the total levels of selenium measured with ICP-MS.

Experimental

Instrumentation

The chromatographic system used for the chiral HPLC studies consisted of a Shimadzu (Columbia, MD, USA) LC-10AT HPLC system equipped with a Shimadzu SPD-10A UV absorbance detector, with the wavelength set to 200 nm. Injections were made using a Rheodyne (Cotati, CA, USA) Model 7725 injection valve with a 20 μ l loop. Separations of the enantiomers of the selenoamino acids were performed using a Daicel Crownpak CR(+) column of dimensions 15 cm \times 4 mm id (Chiral Technologies, Exton, PA, USA). The column was fitted with a thermostating jacket (Chiral Technologies) and water was recirculated through the jacket using a Model 910 recirculating heater/chiller (Fisher Scientific, Fair Lawn, NJ, USA). A guard column, 10 mm \times 4 mm id, with the same stationary phase material was used. UV absorbance (λ = 200 nm) chromatograms were recorded using a Dionex (Sunnyvale, CA, USA) Advanced Computer Interface equipped with AI-450 Chromatography Automation software, version 3.2.1.

For anion exchange chromatography, an ISCO (Lincoln, NE, USA) Model 2350 HPLC pump and an ISCO Model 2360 gradient programmer were used. Samples were injected on to the column using a Rheodyne Model 7725 injection valve with a 20 μ l sample loop. A Hamilton PRP X100 10 μ m anion exchange column was used (Phenomenex, Torrance, CA, USA), of dimensions 250 \times 4.1 mm id. A guard column with the same stationary phase material was also employed.

For ICP-MS detection, a Perkin-Elmer SCIEX, (Ontario, Canada) Elan 6000 was used with a Gem Tipcross-flow nebulizer (Perkin-Elmer) and Rytan spray chamber (Perkin-Elmer). The rf power was 1175 W. The plasma, auxiliary and nebulizer gas flow rates were 15, 1.0 and 0.9 L min⁻¹, respectively. The nebulizer gas flow and ion lens setting were optimized using on-board computer algorithms in the Elan 6000 software. The ion lens voltage was 7.5 V. The outlet of the HPLC column was connected to the liquid sample inlet of the nebulizer using 0.025 mm id PEEK tubing of length 50 cm. Chromatograms were recorded using Perkin-Elmer Turbochrom software, version 6.1.

Reagents and chemicals

All reagents were of analytical reagent grade. The mobile phase for the chiral HPLC studies was perchloric acid (Fisher

Scientific), diluted to 0.10 M using 18 M Ω cm⁻¹ distilled, de-ionized water (Sybron Barnstead, Boston, MA, USA). For anion exchange chromatography, the mobile phase was prepared by combining equal portions of a 40 mM Na₂HPO₄ (Fisher Scientific) solution and a 40 mM NaH₂PO₄ (Fisher Scientific) solution. All mobile phases were filtered through 0.2 μ m nylon membrane filters (Fisher Scientific) and degassed prior to use.

D,L-selenomethionine, D,L-selenoethionine, D,L-selenocystine and L-selenomethionine were obtained from Sigma (St. Louis, MO, USA). Individual stock standard solutions of 1000 μ g mL⁻¹ of each of the selenoamino acids were prepared in the mobile phase and kept for 5 d before discarding. Selenite standard solutions were prepared with sodium selenite (Aldrich, Milwaukee, WI, USA) and selenate standard solutions were prepared with sodium selenate (Aldrich). All standards were filtered through 0.2 μ m nylon membrane filters (Fisher Scientific).

For the gastric enzymolysis digestion, hydrochloric acid (Fisher Scientific), sodium chloride (Fisher Scientific) and pepsin (P7000, Sigma) were used to prepare the selenium supplement samples as described below.

Preparation of selenium supplement samples

Ten commercially available selenium dietary supplements, purchased locally, were analyzed for their selenium content. All of the supplements were in tablet form and the bottles displayed statements as to the source, species and quantity of the selenium in the supplement tablet. A number of the samples reportedly contained selenium from high selenium yeast, which is a high natural source of selenomethionine or of L-selenomethionine. Two samples were multivitamin tablets, which reportedly contained selenium as sodium selenate. Some of the samples did not state the type of selenium in the sample, simply the micrograms of selenium in each tablet. In all cases, our reading of the labels was that they were indicating total elemental selenium.

Seven replicates of each of the tablet types (seven tablets from each bottle) were prepared using a gastric enzymolysis digest method, suitable for the digestion of tablets and capsules, according to the US Pharmacopeia.³⁷ A similar method was used by Crews *et al.*¹⁶ for the preparation of cooked cod samples prior to selenium speciation. Although not the condition of human stomach fluid, this method is intended as an approximate mimic. The method used by the US Food and Drug Administration requires pepsin, NaCl and HCl to simulate gastric juice in the stomach. Sodium chloride (2.0 g) and 3.2 g of pepsin were dissolved in 7.0 mL of hydrochloric acid and sufficient water to make 1000 mL of the simulated gastric juice. This solution had a pH of 1.2. Each selenium supplement tablet was placed in 100 mL of the gastric juice in a plastic screw-topped bottle and placed on a magnetic stirring and heating plate at 37 °C (body temperature) for 4 h. Procedural blanks were also prepared in triplicate, which involved stirring 100 mL of the gastric juice at 37 °C for 4 h with no supplement added. All samples were filtered twice using 0.2 μ m nylon filters (Fisher Scientific) and stored for not longer than 5 d in a refrigerator until required for analysis. The samples were filtered once more prior to injection on to the chromatographic columns.

Seven tablets from each supplement bottle were also digested using concentrated nitric acid so that the total selenium in the samples could be quantified. Each tablet was placed in 30 mL of concentrated nitric acid and heated to boiling on a hot-plate. The samples were then kept at the boiling-point for 2 h, after which they were filtered using 0.2 μ m nylon filters. The acid was then made up to 40 mL using distilled, de-ionized water (Sybron Barnstead).

Results and discussion

Optimization of chiral HPLC separation conditions using UV absorbance detection

Standard solutions of $1000 \mu\text{g mL}^{-1}$ (in the mobile phase) of each of the selenoamino acids were used to evaluate the optimum mobile phase flow rate and column temperature of the Crownpak CR(+) column for the separation of the enantiomers ($20 \mu\text{L}$ injection). UV absorbance detection was used. The range of pH values recommended by the vendor for the operation of this column, with minimal column degradation, is 1–7; the minimum pH of 1 was used. At higher pH, peak resolution decreased. To optimize the resolution for all the peaks, the mobile phase flow rate was initially 0.5 mL min^{-1} and was increased to 1.0 mL min^{-1} after 35 min to decrease the retention time of the final peak. The first six peaks all eluted within 35 min. The separation was sensitive to changes in temperature, therefore the temperature of the column was optimized. Fig. 1 shows that the resolution of all seven peaks was obtained at the optimum temperature of 22°C . At 22°C , peaks 5 and 6 approach baseline resolution and peaks 2 and 3 are completely baseline resolved. It should be noted that the D-enantiomers elute before the L-enantiomers in the Crownpak CR(+) column.

Peak reproducibility values, in terms of peak area and retention time, were measured with UV detection at a column temperature of 22°C (Table 1). The relative standard deviations (RSDs), based on three replicates, were well below 5% for all the enantiomeric species measured. Absolute limits of detection

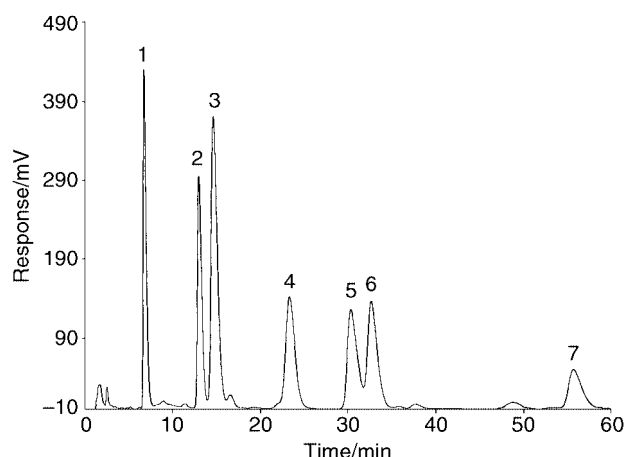


Fig. 1 HPLC separation at 22°C of D,L-selenocystine, D,L-selenomethionine and D,L-selenoethionine enantiomers using UV absorbance detection at 200 nm with $20 \mu\text{L}$ injection of a $1000 \mu\text{g mL}^{-1}$ standard. Mobile phase, 0.1 M HClO_4 (pH 1), 0.5 mL min^{-1} for 35 min then increased 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.

for all the species, measured by calculating three times the standard deviation of the blank and dividing by the slope of the calibration graph, are also given in Table 1.

Chiral HPLC with ICP-MS detection

The chromatographic eluent, after passing through the UV detector of the Shimadzu chromatograph, was pumped through PEEK tubing to the nebulizer of the ICP-MS. The original $1000 \mu\text{g mL}^{-1}$ standards of the selenoamino acids were serially diluted for the ICP-MS studies. To investigate the effect of the standard matrix, the standards were prepared in both water and the mobile phase. Fig. 2 shows the enantiomeric separation of the three selenoamino acids at $10 \mu\text{g mL}^{-1}$ concentration (as the selenoamino acid) prepared in mobile phase. The baseline becomes unstable when standards are prepared in water. This is due to the difference in pH of the mobile phase (1) and the pH of the standards prepared in water (5). It should also be noted that the signal-to-noise ratio of the ICP-MS was also greater when using HClO_4 . This may be due to a sample transport phenomenon when the samples are in an acid matrix.³⁶

The reproducibility of the separation with ICP-MS detection is given in Table 1 in terms of both peak area and peak retention time. The RSDs were very good. This indicates that transport of the analyte through the sample introduction system of the ICP-MS does not negatively affect the reproducibility compared with UV absorbance detection where no nebulizer interface is required. The calibration curves for each of the enantiomers were linear over three orders of magnitude of concentration. The absolute limits of detection for ICP-MS detection are also given in Table 1. It can be seen that the absolute limits of detection afforded using ICP-MS detection are lower than for UV absorbance detection by factors ranging from *ca.* 40 to 400, highlighting the advantage of improved sensitivity with ICP-MS as a chromatographic detector for these separations.

Analysis of selenium supplement samples using HPLC

Another advantage of ICP-MS is its element specificity. Fig. 3 shows a procedural blank for simulated gastric juice when using UV detection. Fig. 4(a) and (b) show the UV and ICP-MS chromatograms obtained using each method of detection for a tablet of one of the selenium yeast supplements. The UV absorbance chromatograms in Fig. 3 and 4(a) are virtually identical, showing a large, unretained peak followed by several smaller peaks with retention times that do not coincide with the retention times of the selenoamino acid species as shown in Fig. 1. It is likely that several of the peaks shown in Fig. 3 and 4(a) may be attributed to UV absorbing species present in the simulated gastric juice. Other peaks in Figure 4(a) may be attributed to excipients in the selenium supplement which are retained by the column and also absorb UV radiation. However, on their own, they provide little useful analytical information.

Table 1 Reproducibility studies for the chiral separation of three selenoamino acids along with absolute limit of detection values utilizing UV absorbance detection and ICP-MS detection

Amino acid	Retention time/min		RSD of retention time (%) ^a		RSD of peak area (%) ^a		Absolute limit of detection/ng ^b	
	UV	ICP-MS	UV	ICP-MS	UV	ICP-MS	UV	ICP-MS
L-Selenocystine	6.96	7.41	0.9	1.3	1.6	1.4	45.4	0.11
L-Selenomethionine	13.2	13.8	1.0	0.7	3.1	1.5	34.5	0.31
Meso-Selenocystine	14.8	15.4	1.4	0.4	3.7	3.8	47.1	0.42
D-Selenocystine	23.3	24.1	1.4	0.7	3.6	1.0	42.0	0.74
D-Selenomethionine	30.5	31.5	1.8	0.1	2.5	0.7	36.6	0.99
L-Selenoethionine	32.5	33.9	0.6	0.4	1.9	1.3	43.1	0.24
D-Selenoethionine	55.3	56.9	1.5	0.2	3.5	2.1	37.4	0.14

^a $n = 3$. ^b Calculated for a $20 \mu\text{L}$ injection volume.

The chromatogram shown in Fig. 4(b) obtained with ICP-MS is simpler and more informative since only species at the selenium isotope, m/z 82, are detected. Peaks 3 and 4 are attributable to D- and L-selenomethionine, respectively, based on comparison of the retention times shown in Fig. 2.

The total amount of selenium in each of the tablets was determined by nitric acid digestion and digestion in simulated gastric juice. In addition, each selenium supplement sample was analyzed using chiral HPLC with UV and ICP-MS detection in tandem (ICP-MS immediately following UV detection). The type of selenium species and quantity for each of the seven tablets of ten sample types are summarized in Table 2 along with the total selenium values. RSD values also are given for all analyses. Generally, for the tablets with selenoamino acids, the RSDs are poorer than indicated in Table 2 for the standards, suggesting poorer tablet to tablet reproducibility.

Both multivitamins exhibited unretained species when using chiral HPLC with ICP-MS detection. The presence of unretained peaks indicates that the species actually present in the tablet cannot be attributed to an amino acid, but rather to some other selenium species that does not contain a primary amine group. For samples containing unretained species using chiral HPLC with ICP-MS detection, species identification was

confirmed using an anion exchange chromatographic method based on that developed by Hagege *et al.*³⁸ Anion exchange chromatography confirmed that both multivitamins contained selenium in the form of selenate, which agrees with the information on the package labels. Total selenium amounts determined by HPLC-ICP-MS and by both digestion methods agree reasonably well with total selenium as stated on the bottle label.

Anion exchange chromatography showed the selenium in 'selenium tablet 1' was in the form of selenite. The amount of selenium determined by anion exchange-ICP-MS was significantly lower than the value reported on the bottle label. The unretained peak observed with chiral HPLC also was very small and approached the limit of quantification. However, the total selenium amounts determined using both digestion methods are reasonably close to the values stated on the bottle label. The reason for this discrepancy is not clear. The product label provides no information concerning the form of the selenium in the supplement.

Two yeast-free selenium supplements were analyzed. The selenium amounts determined by both digestion methods agree reasonably well with the amounts of selenium reported on the bottle labels. The 'yeast free Se 1' sample was reported to contain selenium in the form of L-selenomethionine, but no selenomethionine was detected in the chiral HPLC analysis. Instead, 160 μg of selenium in the form of selenate were detected. The 'yeast free Se 2' did contain the same amount of selenium reported on the bottle label in the form of L-selenomethionine, and no other forms of selenium were detectable in the sample. This is shown in Fig. 5(a) and

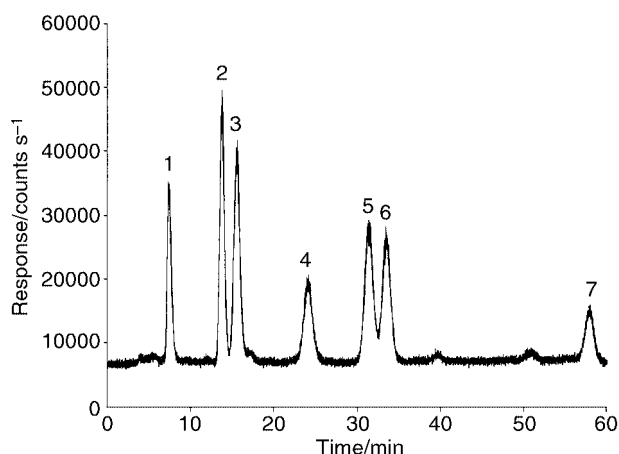


Fig. 2 Separation of selenoamino acids prepared with 0.1 M HClO_4 as the sample matrix using ICP-MS detection. Mobile phase; 0.1 M HClO_4 (pH 1), 0.5 mL min^{-1} for 35 min then increased to 1.0 mL min^{-1} . Peaks: 1 = L-selenocystine; 2 = L-selenomethionine; 3 = *meso*-selenocystine; 4 = D-selenocystine; 5 = D-selenomethionine; 6 = L-selenoethionine; and 7 = D-selenoethionine. Injection of 20 μL of a 10 $\mu\text{g mL}^{-1}$ concentration standard.

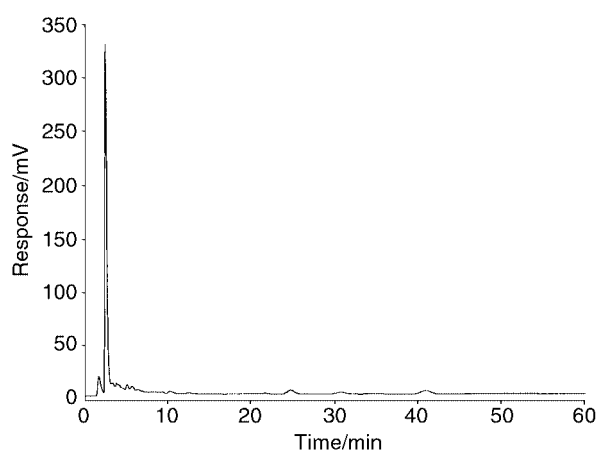


Fig. 3 Analysis of the procedural blank using UV absorbance detection at 200 nm. Mobile phase, 0.1 M HClO_4 (pH 1), 0.5 mL min^{-1} for 35 min then increased to 1.0 mL min^{-1} .

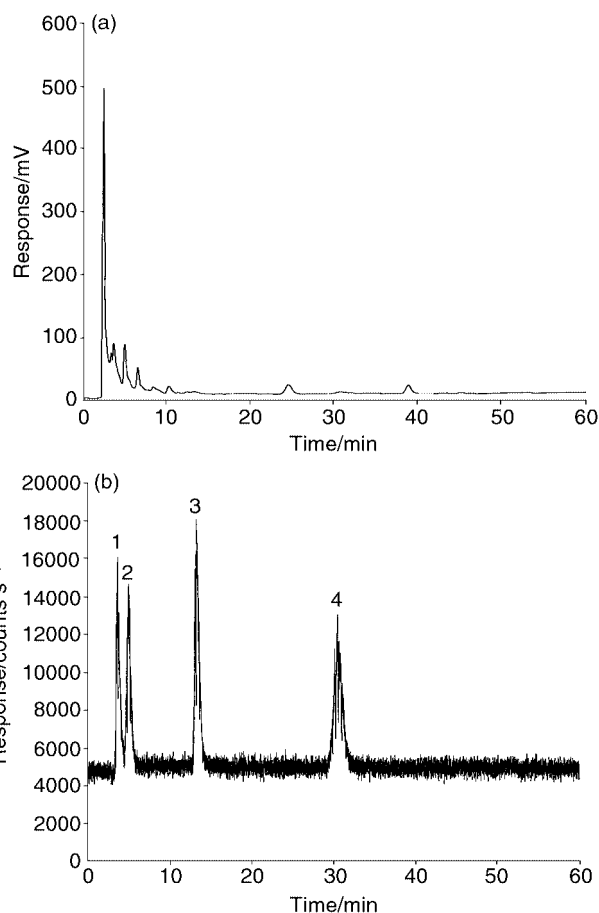


Fig. 4 Comparison of (a) UV absorbance detection at 200 nm and (b) ICP-MS detection for the analysis of a selenium supplement tablet. Mobile phase, 0.1 M HClO_4 (pH 1), 0.5 mL min^{-1} for 35 min then increased to 1.0 mL min^{-1} . Peaks: 1 and 2 may be attributed to another selenoamino acid; 3 = L-selenomethionine, 4; D-selenomethionine.

illustrates clearly the selectivity that can be attained from a complex sample when a selective detector is utilized in tandem with a highly selective chromatographic method. Fig. 5(b) presents the UV chromatogram for the same separation and is given to show some enhancement with the UV detector over the procedural blank, yet with similarities remaining. The comparison of Fig. 5(a) and (b) illustrate best in this work the clear selectivity advantage ICP-MS has for this type of complex sample. Moreover, with UV detection alone, the analysis would be highly compromised if not impossible.

Five selenium yeast supplements were analyzed. Nitric acid and simulated gastric digestion data for total selenium agree reasonably well with the values on the bottle label for the first three selenium yeast samples shown in Table 2. 'Se yeast 4' and 'Se yeast 5' both have lower selenium concentrations in the gastric juice and nitric acid digests than was stated. The close agreement between selenium amounts determined by digestion and selenium values reported on the labels for other samples indicates that the actual selenium concentration in the tablets for Se yeast 4' and 'Se yeast 5' may be lower than reported on the bottle label. Inorganic selenium was found to be present in 'Se yeast 1' and 'Se yeast 2' samples.

For all yeast samples, the concentration of selenium determined using chromatographic methods was significantly lower than the selenium concentration determined by analyzing the digested tablets directly by ICP-MS. This same phenomenon is not consistently observed when selenium derived from other sources is analyzed. The discrepancy in selenium concentrations is apparently related to the nature of selenium derived from selenium enriched yeast. The selenoamino acids coming from yeast are expected to be protein bound. When the tablets are subjected to an enzymolysis (simulated gastric digestion), the selenoamino acids are liberated. Pepsin from porcine stomach mucosa was used in this study to perform the simulated gastric digestion. Pepsin is a type of protease that is more specific in cleaving peptide bonds than other proteases. If the peptide bonds are not all cleaved, some of the selenoamino acids may still be protein bound and not available for the separation, resulting in a diminished amount of selenoamino acids detected. This will be studied further using different enzymes and alternative chromatographic methods as part of an ongoing study.

Conclusions

A Daicel Crownpak CR(+) crown ether column was used to perform simultaneous enantiomeric separations of D,L-seleno-

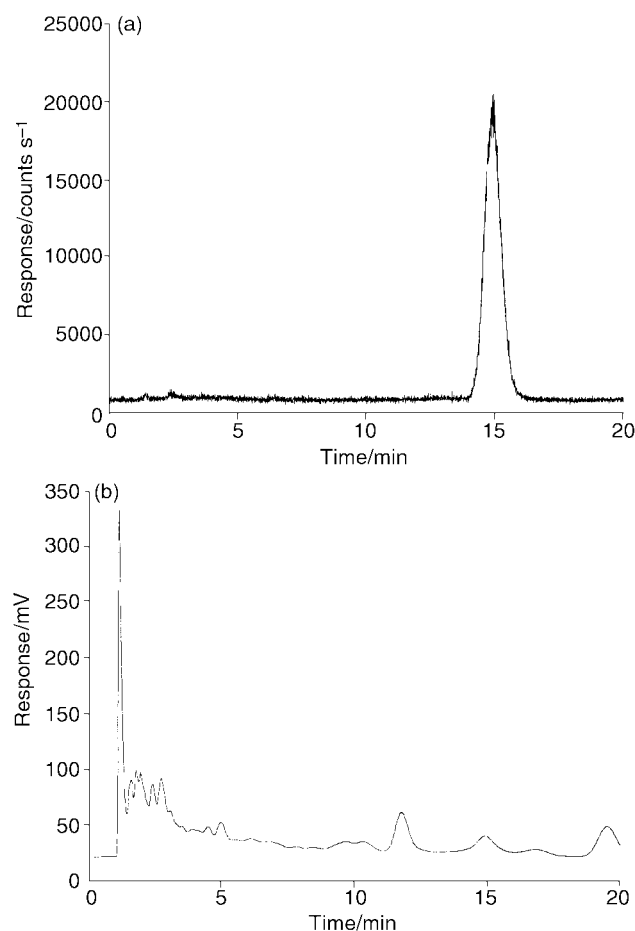


Fig. 5 (a) ICP-MS chromatogram for supplement 'Yeast free Se 2' showing a single peak attributable to L-selenomethionine; (b) UV chromatogram for supplement 'Yeast free Se 2'.

Table 2 Results from selenium supplement analyses^a

Sample		Total Se/ μ g		D-Seleno-methionine/ μ g as Se	L-Seleno-methionine/ μ g as Se	Selenate/ μ g as Se	Selenite/ μ g as Se	Stated amount/ μ g (plus other label indications)
		Nitric acid digestion	Gastric digestion					
Multivitamin 1	Mean	26.4 \pm 1.5	28.3 \pm 2.7	ND ^b	ND	31.3 \pm 2.0	ND	20 as selenate
	RSD (%)	5.5	9.5			6.5		
Multivitamin 2	Mean	198 \pm 19	186 \pm 8.1	ND	ND	188 \pm 18	ND	200 as selenate
	RSD (%)	9.6	4.4			9.3		
Selenium tablet 1	Mean	50.7 \pm 7.5	42.7 \pm 2.0	ND	ND	ND	9.27 \pm 0.80	50 as Se
	RSD (%)	15	4.7				8.6	
Yeast free Se 1	Mean	197 \pm 26	217 \pm 13	ND	ND	160 \pm 5.0	ND	200 as L-Selenate methionine
	RSD (%)	13	5.9			3.0		
Yeast free Se 2	Mean	86.5 \pm 4.6	84.4 \pm 3.6	ND	97.1 \pm 9.7	ND	ND	100 as L-Selenate methionine
	RSD (%)	5.3	4.3		10			
Selenium yeast 1	Mean	104 \pm 16	89.2 \pm 23	ND	ND	ND	16.0 \pm 1.5	100 from yeast
	RSD (%)	16	25				9.5	
Selenium yeast 2	Mean	99.1 \pm 11	101 \pm 3.3	ND	ND	68.7 \pm 3.2	ND	100 from Se yeast
	RSD (%)	11	3.3			0.51		
Selenium yeast 3	Mean	248 \pm 56	187 \pm 6.4	ND	8.95 \pm 0.74	ND	ND	200 from yeast
	RSD (%)	23	3.4		8.3			
Selenium yeast 4	Mean	135 \pm 16	108 \pm 9.4	5.32 \pm 2.2	8.67 \pm 2.8	ND	ND	200 from yeast
	RSD (%)	12	8.8	42	33			
Selenium yeast 5	Mean	103 \pm 17	104 \pm 6.9	ND	11.9 \pm 1.2	ND	ND	200 from yeast
	RSD (%)	18	6.6		9.9			

^a Results are mean \pm s. ^b ND = none detected.

cystine, D,L-selenomethionine and D,L-selenoethionine with success. The optimum separation temperature was 22 °C and it was found that a fluctuation of the temperature by ± 1 °C resulted in a decrease in resolution of some of the chromatographic peaks.

UV absorbance and ICP-MS detection were compared and, not surprisingly, it was found that ICP-MS provided lower detection levels for the determination of enantiomers of the selenoamino acids.

The additional advantages of ICP-MS detection are shown with the nutritional supplement samples. UV detection provided little useful analytical information for these samples in the simulated gastric juice, whereas ICP-MS confirmed the presence of selenium in the samples and was superior in terms of sensitivity and selectivity. Only the multivitamins and 'Yeast free Se 2' accurately report Se species and amounts as indicated by the bottle label. Although we do not claim that this relatively small sampling is sufficient for the statistical sampling protocol necessary to draw conclusions about nutritional supplements, there is at least an inclination to study these substances further, as yet unregulated in the USA and ever growing in popularity.

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