ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 507–514

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Capillary gas chromatography inductively coupled plasma mass spectrometry (CGC-ICPMS) for the enantiomeric analysis of D,L-selenomethionine in food supplements and urine

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Received 4 April 2001; received in revised form 22 June 2001; accepted 22 June 2001

Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

Capillary gas chromatography inductively coupled plasma mass spectrometry (CGC-ICPMS) was applied to the determination of D- and L-selenomethionine in food supplements and in urine. Derivatization was performed with ethylchloroformate (ECF) offering the advantage that the reaction can be carried out in aqueous medium i.e. urine. The derivatives were separated on the chiral stationary phase (CSP) Chiralsil-L-Val. The method was validated with D- and L-seleno-ethionine as internal standard (IS) and the linearity for a seven point calibration from 12.5 pg to 2.5 ng per enantiomer was excellent (R^2 0.9997). Repeatability of injection ($n = 3$) was $< 1.8\%$. The limit of detection (LOD) and quantification (LOQ) were 4 and 12 pg, respectively. Food supplements presently on the market contain L-selenomethionine for at least 90%. Repeatability of the whole procedure ($n = 6$) was tested on one L-selenomethionine formulation and was 3.8 (R.S.D.%). Data for urine samples after a daily intake of L-selenomethionine or the racemate D,L-selenomethionine corresponding to 100 μg selenium indicate that the D-enantiomer is not metabolized.

Keywords: Capillary gas chromatography inductively coupled mass spectrometry (CGC-ICPMS); D,L-selenomethionine; Chiral separation; Food supplements; Urine

1. Introduction

Nowadays, in addition to the normal nutritive intake, food supplements are more and more taken to provide for the required daily quantity of essential nutrients, such as vitamins, minerals, amino acids, etc. The variety of available products has increased significantly in recent years and the composition of these food supplements, often taken on a personal base, i.e. without actual medical assistance, is not always or only partly known.

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00576-3

A common constituent of food supplements is selenium, often called the wonder mineral, and Se is present either in the organic (selenomethionine, selenium-yeast) or in the inorganic form (sodium selenite). The nutritional bioavailability [1–3] and toxicity [1,4,5] of Se are strongly influenced by its chemical form. Selenomethionine and selenized yeast are considered less toxic than inorganic Se forms while their bioavalability is higher [6].

Selenomethionine, however, is chiral and the individual enantiomers, from which the L-enantiomer is natural, can play a different role in our organism. Sources have mentioned that the D-enantiomer is metabolized to inorganic Se that is only one-fifth as bioavailable as L-selenomethionine and that is toxic at too high levels [7].

In an earlier study [8], we have noted that some formulations, commercially available at that time in drug stores, contained synthetic selenomethionine, i.e. racemate or 50% L and 50% D. Selenomethionine was extracted from the pills with 0.1 N hydrochloric acid and after a one-step derivatization with ethylchloroformate (ECF) analyzed with capillary gas chromatography coupled to atomic emission detection and monitoring at the 196 nm emission line. Selenomethionine chiral speciation in yeast and parental solutions by capillary gas chromatography inductively coupled mass spectroscopy (CGC-ICPMS) has recently been described [9]. *N*-trifluoroacetyl(TFA)-*O*-iospropyl derivatives were prepared and this required a two step reaction and working under dry conditions.

In this contribution, CGC-ICPMS is evaluated and validated for the determination of D- and L-selenomethionine in food supplements, presently on the market, applying the one-step ECF reaction. The developed method was applied to monitor L and D residues in urine after selenomethionine (pure L or racemate) intake. Emphasis in this paper is on the analytical methodologies.

2. Experimental

2.1. Chemicals

L-Selenomethionine, racemic D,L-selenomethionine, D,L-seleno-ethionine used as internal standard (IS), and the derivatization reagent ECF were obtained from Acros Organics (Geel, Belgium). Ethanol, pyridine and chloroform were purchased from Sigma-Aldrich (Bornem, Belgium). Milli-Q water was prepared by purification and deionization of tap water in a Milli-Q plus water system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

Different food supplements containing selenomethionine were obtained from an American drug store. To a quantity corresponding to 50 μg Se, 5 ml 0.1 N HCl and 5-ml chloroform were added [8]. The sample was placed in an ultrasonic bath for 10 min followed by centrifugation at 1500 rpm for 15 min. Hundred microliters of the aqueous fraction was transferred to a teflon vial and derivatized with ECF according to Husek [10]. Two milliliters of the solvent mixture (water:ethanol:pyridine in ration 60:32:8) and 200 μl ECF were added and the tube was vigorously shaken on a vortex for 60 s. The *N*-ethoxycarbonyl-*O*-ethyl ester derivatives were extracted from the mixture using 1 ml of chloroform. One microliter of this solution was injected splitless (see further). For selenized yeast samples, the same procedure was applied after acidic hydrolysis with 6 N HCl [11].

Urine samples were collected from three volunteers who took 100 μg Se at 1.00 pm. Two persons took L-selenomethionine while the third one the racemate. Approximately 50 ml urine was collected at 6.00 pm. To 1 ml urine sample, 4 μl of a water solution of the IS solution (10 mg/l D, L-seleno-ethionine) was added and the sample was then derivatized as described for the food supplements.

2.3. CGC-ICPMS

The samples were analyzed using an HP 5890 series II GC coupled to a 4500 benchtop ICPMS (Agilent Technologies, Waldbronn, Germany). One microliter was injected splitless (260 °C) with a splitless time of 2 min, onto a Chirasil-L-Val capillary column (Alltech, Lokeren, Belgium) 25 m in length, 0.25 mm internal diameter and 0.16 μm film thickness. The oven was programmed from 40 °C (1 min) to 135 °C at 40 °C/min and then to

165 °C (5 min) at 2 °C/min. Hydrogen spiked with 500 ppm xenon [12] was used as carrier gas at 2 ml/min. The temperature of the home-made transfer line was set at 250 °C [12,13].

ICPMS detection was performed on Se^{82} (dwell time 200 ms), which gave the best signal to noise ratio. RF power and sampling depth were set at 1300 W and 5.8 mm, respectively. Argon carrier gas flow was set at 1.6 l/min while the make-up and plasma gas flow were 1.0 and 15 l/min, respectively.

3. Results and discussion

Initial experiments were performed on standard amino acids with the one-step ECF derivatization and the classical two-step derivatization with isopropanol (IPA) and trifluoroacetic acid anhydride (TFA) [9]. Although the *N*-trifluoroacetyl(TFA)-*O*-isopropyl derivatives showed a somewhat higher resolution than the *N*-ethoxycarbonyl-*O*-ethyl ester derivatives, 1.8 and 1.4, respectively, for nearly the same analysis times, the ECF derivatization was preferred, because of its simplicity and operation in aqueous medium. The IPA/TFA procedure is time-consuming and needs several fractionation and evaporation steps. Fig. 1 shows the separation of D,L-selenomethionine (25 ppb) and D,L-seleno-ethionine (250 ppb). On Chirasil-L-Val, the L-enantiomer elutes after the D-enantiomer [8,9]. A calibration curve was constructed based on the responses of seven aqueous standard solutions in a concentration range of 25 ppb–5 ppm D,L-selenomethionine keeping the IS concentration constant at 250 ppb (Fig. 2). The slope and the intercept of the calibration curve were 0.0032 and 0.1734, respectively, with a linearity factor of 0.9997. The repeatability of injection ($n = 3$) was tested and was 1.85 (R.S.D.%). The limit of detection (LOD)

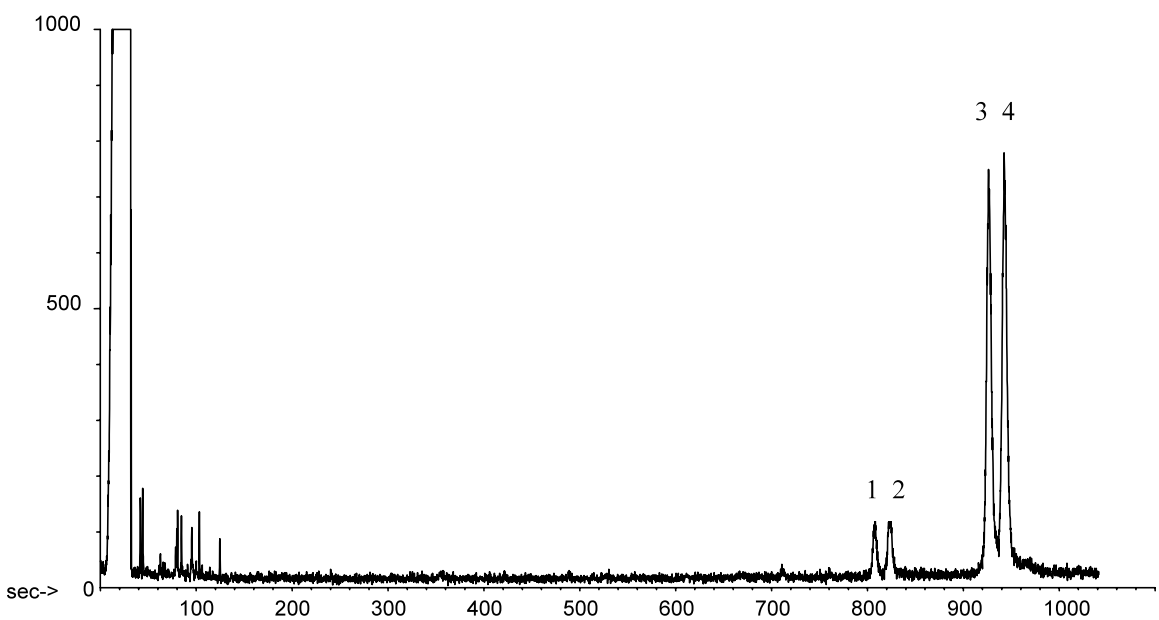

Fig. 1. CGC-ICPMS chromatogram at Se 82 for a standard mixture of the racemates of D,L-selenomethionine (25 ppb) and D,L-seleno-ethionine (250 ppb) as the *N*-ethoxycarbonyl-*O*-ethyl ester derivatives on Chirasil-L-Val. Conditions see text. Peaks: (1) D-selenomethionine; (2) L-selenomethionine; (3) D-seleno-ethionine; (4) L-seleno-ethionine.

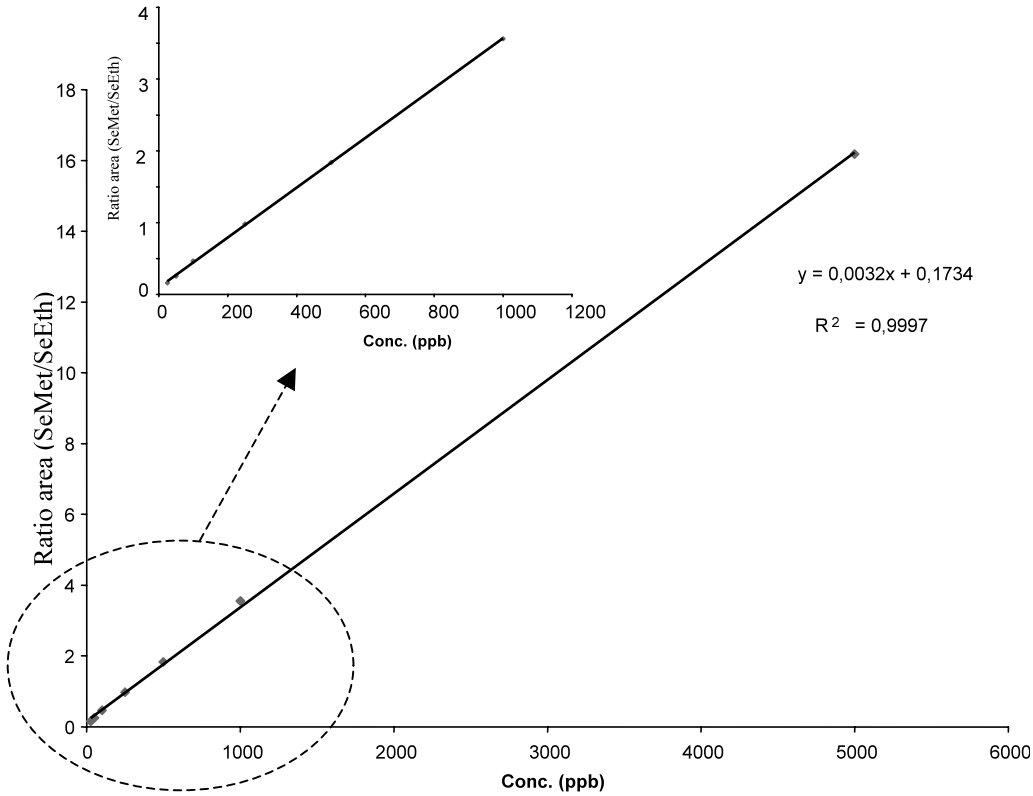

Fig. 2. Seven point calibration graph from 25 ppb to 5 ppm.

defined as the amount corresponding to a signal (S) three times the noise level (N) was 4 pg for each enantiomer (corresponding to 4 ppb for a 1 μl injection) while the limit of quantitation (LOQ) defined at S/N 10 is approximately 12 pg for each enantiomer (corresponding to 12 ppb for a 1 μl injection). Fig. 1 in fact is very close to the LOQ. It should be noted that with present state-of-the-art capillary GC aliquots of 5 μl via splitless injection or up to 100 μl with programmed temperature vaporization (PTV) injection operated in the solvent venting mode can be introduced into the column increasing the sensitivity of the method. However, such high sensitivities were not needed for the present study.

Fig. 3 shows some representative chromatograms namely of a L-selenomethionine formulation (100 μg Se per pill according to the manufacturer) (A and B), of a selenized yeast formulation (C) and of a formulation with the only specification 50 μg per capsule organic Se (D). From Fig. 3A, it can be deduced that this product only contains the natural L-enantiomer. Adding the IS only 91% of the selenium indicated on the package could be measured (Fig. 3B) while the recovery of the internal standard as ascertained by comparison of a SI directly was 100%. Repeatability of the procedure ($n = 6$) was 3.8 (R.S.D.%). The pills had a very strong 'garlic' smell that was elucidated by CGC–MS as caused by dimethyldiselenide. Most probably decomposition is responsible for the lower quantity. In the yeast formulation (Fig. 3C) small traces of the D-enantiomer (ca. 1%) were detected. This is in agreement with [9]. It has been reported that D-amino acids in food products can originate from yeast [14]. It is also clear from the chromatogram that several other selenium-containing species are present. In a formulation with unknown composition the presence of selenized

yeast was elucidated (similar to Fig. 3C) but upon analysis without acidic hydrolysis, the chromatogram in Fig. 3D was obtained. Based on extrapolation of the retention characteristics of selenomethionine (C3) and seleno-ethionine (C4), this enantiomeric pair most probably is selenocysteine. Their origin is unknown and this chromatogram seems extremely rich on unknown selenium-containing compounds.

The urine samples (Fig. 4) of the persons who took pure L-selenomethionine did not contain residues of the seleno amino acid (Fig. 4A) while in the urine of the person taking D,L-selenomethionine racemate, the L-enantiomer is absent while the D-enantiomer apparently is not metabolized in our organism (Fig. 4B). Remarkable in both chromatograms is the extremely high selectivity of the CGC-ICPMS combination. ECF is a

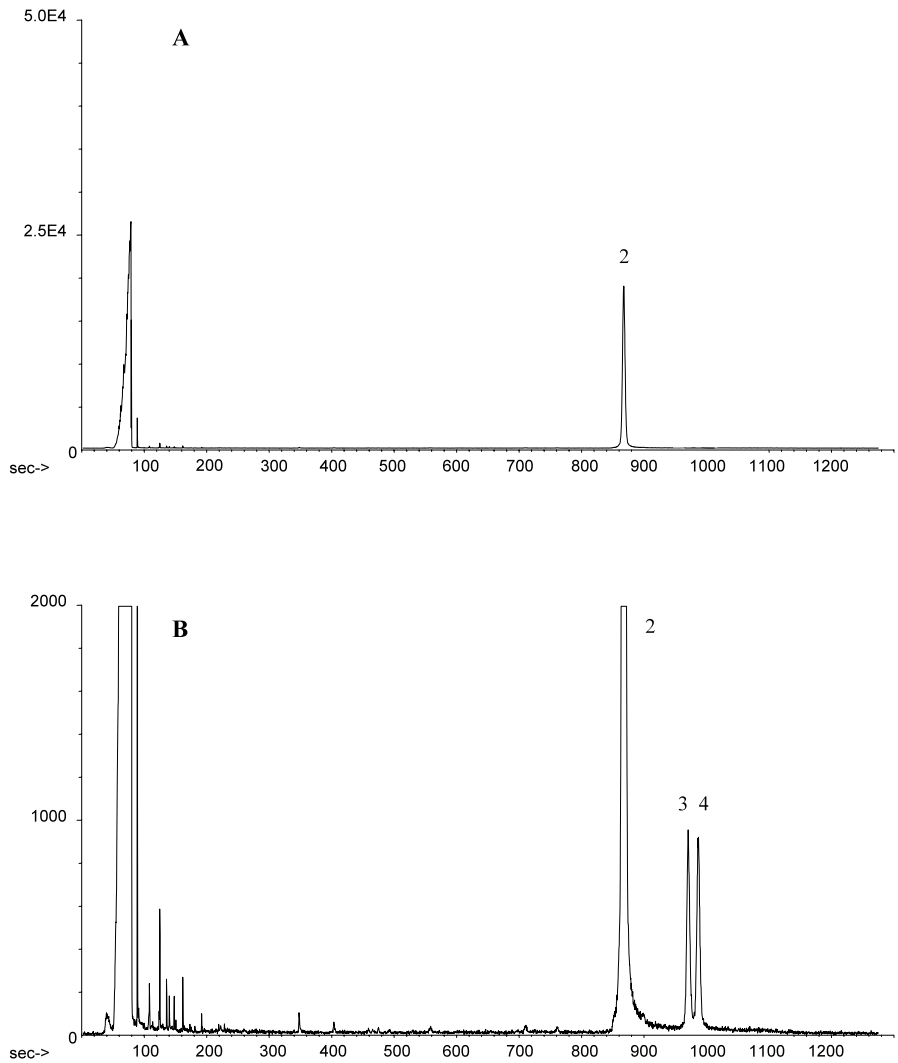

Fig. 3. CGC-ICPMS chromatograms of different food supplements. (A) L-selenomethionine formulation. (B) L-selenomethionine formulation (A) with IS addition. (C) Selenized yeast after acid hydrolysis. (D) Unknown formulation without acid hydrolysis. Conditions see text. Peaks as in Fig. 1 and (5) D-selenocysteine; (6) L-selenocysteine.

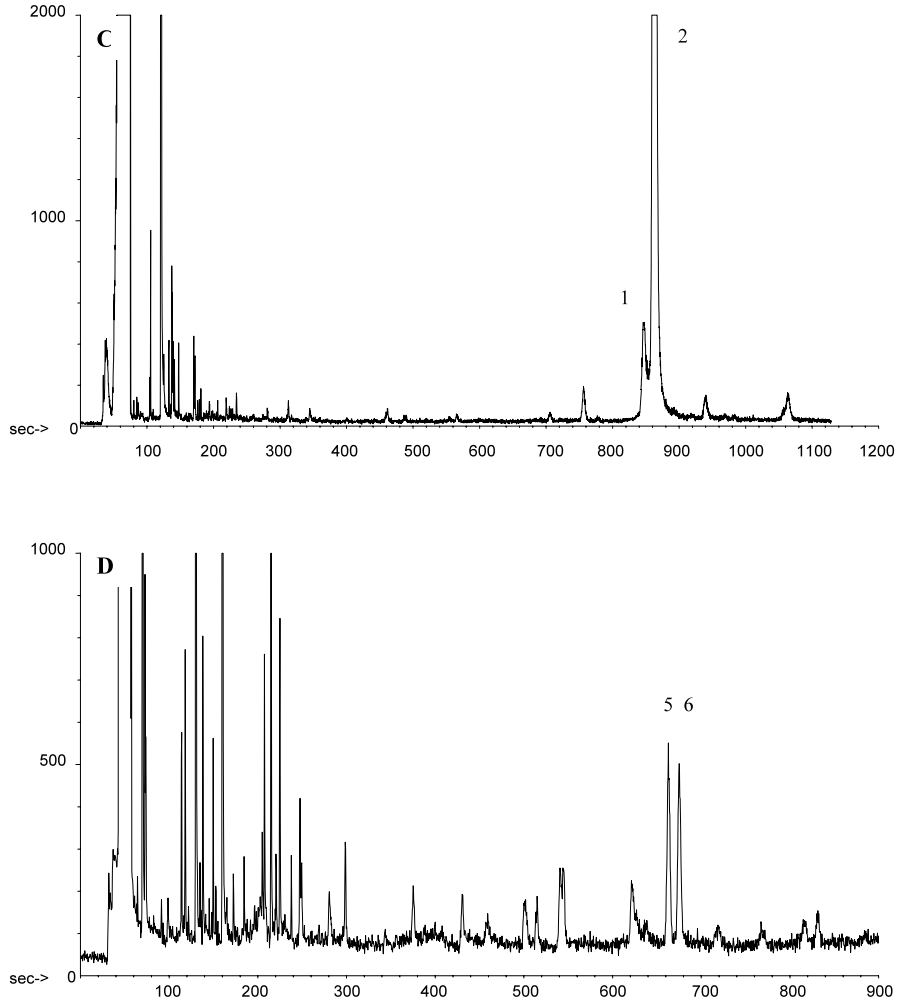

Fig. 3. (*Continued*)

powerful derivatization reagent for other functionalities as well [10] and a urine sample give rise to a complex chromatogram with universal detection system such as mass spectrometry (MS) and flame ionization detection (FID).

The technology described is presently applied to an in depth and quantitative study of the metabolisms of L, D and D,L-selenomethionine. Other techniques used in this study are ICPMS for trace selenium analysis and solid phase microextraction (SPME)-CGC-ICPMS/MS for monitoring the volatile selenium metabolites methylhydrogenselenide (CH_3SeH), dimethylselenide (CH_3Se-CH_3), dimethyldiselenide (CH_3–Se–Se–CH_3) and the non-volatile trimethylselenium cation (($(CH_3)_3$-Se^+) after derivatization with sodium tetraethylborate [15].

4. Conclusion

CGC-ICPMS on a chiral stationary phase (CSP) (Chirasil-L-Val) is a powerful technique for the elucidation of the chirality of selenomethionine in

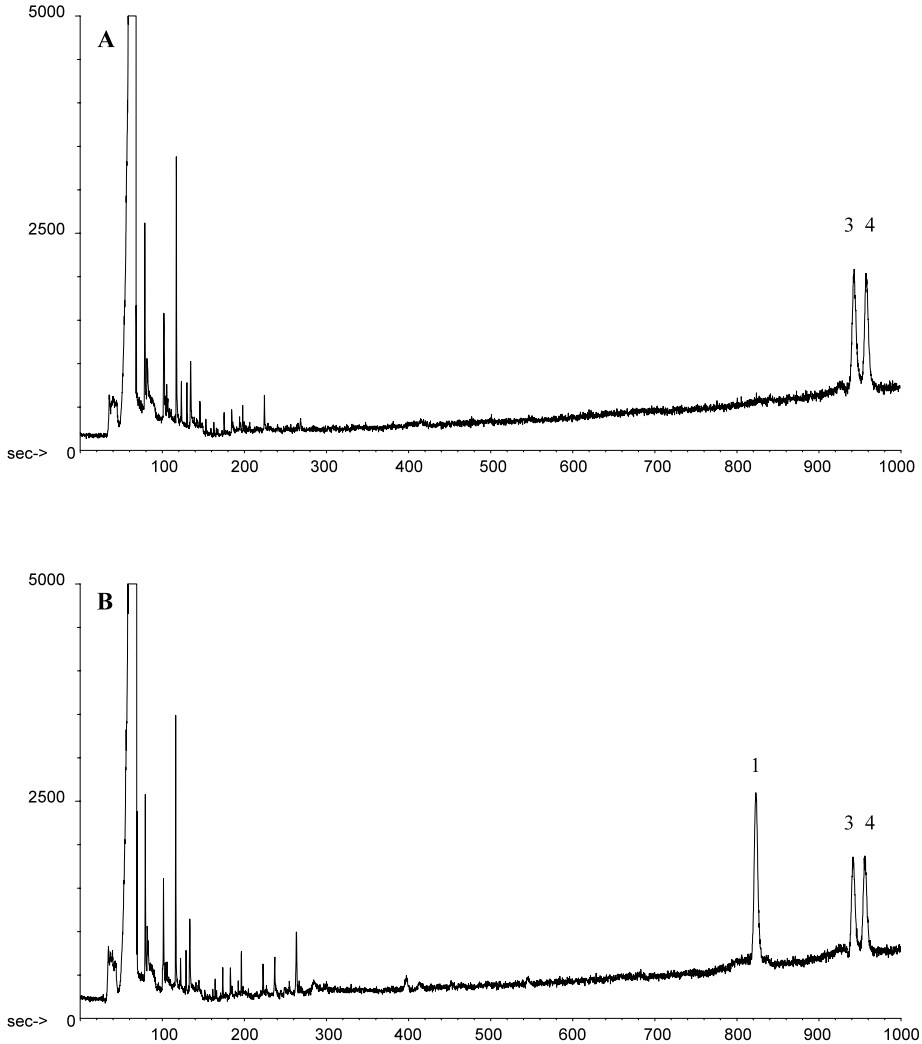

Fig. 4. CGC-ICPMS chromatograms of urine after intake of L-selenomethionine (A) and D,L-selenomethionine (B). Conditions see text. Peaks as in Fig. 1.

food supplements. The amino acids are derivatized in a one-step reaction with ECF. This procedure allows to derivatize the target solutes in aqueous solution e.g. urine. Upon intake of L-selenomethionine (the natural enantiomer) no residues are detected in urine, while by intake of the racemate D,L-selenomethionine (synthetic product) the D-enantiomer is recovered indicating that this enantiomer is not or only partly metabolized in our organism. This should become clear from studies presently carried out.

References

[1] L.H. Foster, S. Sumar, Crit. Rev. Food. Sci. Nutr. 37 (1997) 211.
[2] S.J. Fairweather-Tait, Eur. J. Clin. Nutr. 51 (1997) S20.
[3] A.J. Butler, C.D. Thomson, P.D. Whanger, M.F. Robinson, Am. J. Clin. Nutr. 53 (1991) 748.
[4] C.C. Willhite, W.C. Hawkes, S.T. Omaye, W.N. Choy, D.N. Cox, M. Curierski, J. Food Chem. Toxicol. 30 (1992) 903.
[5] G.H. Heinz, L.J. Hoffman, L.J. LeCaptain, Arch. Environ. Contam. Toxicol. 30 (1996) 93.
[6] A.M. Smith, M.F. Picciano, J. Nutr. 117 (1987) 725.

[7] C.D. Thomson, Am. J. Clin. Nutr. 36 (1982) 24.
[8] F. David, H. Billiet, P.T.K. Trang, P. Sandra, in: P. Sandra (Ed.), Proceeding of the 18th International Symposium on Capillary Chromatography, Hüthig Verlag, Heidelberg, Germany, 1996, p. 1281.
[9] S. Perez Méndez, M. Montes Bayón, E. Blanco González, A. Sanz-Medel, J. Anal. At. Spec. 14 (1999) 1333.
[10] P. Husek, J. Chromatogr. B 717 (1998) 57.
[11] R. Mateo Castro, M.T. Domenech Carbo, V. Peris Martínez, J.V. Gimeno Adelantado, F. Bosch Reig, J. Chromatogr. A 778 (1997) 373.
[12] L. Moens, T. De Smaele, R. Dams, P. Van Den Broeck, P. Sandra, Anal. Chem. 69 (1997) 1604.
[13] T. De Smaele, L. Moens, P. Sandra, R. Dams, Mikrochim. Acta 130 (1999) 241.
[14] H. Bruckner, M. Langer, M. Lupke, H.T. Westhauser, J. Chromatogr. A 697 (1995) 229.
[15] C. Devos, K. Sandra, P. Sandra, in preparation.