Acta Univ. Sapientiae, Alimentaria, **6** (2013) 45–51

# Determination of seleno-amino acids by ion-exchange column chromatography and high-performance liquid chromatography (Preliminary study)

J. Csapó
email: csapojanos@sapientia.siculorum.ro,
csapo.janos@gmail.hu

Cs. Albert
email: albertcsilla@sapientia.siculorum.ro

Sapientia – Hungarian University of Transylvania,
Faculty of Technical and Social Sciences,
Department of Food Science
Piaţa Libertăţii 1, 530104 Miercurea Ciuc, Romania

**Abstract.** The biological role of selenium was discovered in the second half of the last century. It was established that selenium was a part of the antioxidant system of the organism, and it protected the organism from the attack of the harmful free radicals. The researches made it clear that the organically bound selenium, like selenomethionine (SeMet) and selenocysteine (SeCys), had an especially important role. It is very important for experts dealing with both food science and feeding stuffs to determine the concentration of these two substances in foods and feeding stuffs. We have no information on whether the enantiomers of these two seleno-amino acids have ever been examined before by anyone else; therefore, we have elaborated new methods and amended old ones, respectively, for the exact determination of these compounds by

**Keywords and phrases:** selenomethionine, selenocysteine, determination of seleno-amino acids, ion-exchange column chromatography, IEC, high-performance liquid chromatography, HPLC.

ion-exchange column chromatography (IEC) and high-performance liquid chromatography (HPLC). In the first step, we carried out the separation of the seleno-amino acids along with protein-components amino acids by ion-exchange column chromatography. In the next step, we attempted the separation of the oxidized derivatives of these amino acids by IEC, utilizing our experiences gained during the determination of methionine (Met) and cysteine (Cys) in oxidized form. Afterwards, we tried to increase the sensibility by applying HPLC after precolumn derivatization with OPA/2-mercaptoethanol and OPA/2-mercaptoethane sulphonic acid, respectively, as the seleno-amino acids are contained in the biological samples, in a several orders of magnitude lower concentration as compared to the protein-component amino acids. For the separation of the seleno-amino acid enantiomers, the OPA/TATG derivatization method was used, which we applied for amino acid enantiomers beforehand, but derivatization with 1-(9-fluorenyl)-ethyl chloroformate (FLEC) was also attempted.

# 1 Introduction

About 1930, selenium was still considered a toxic element; however, by 1943, it had already been proven that its presence in the living organism is essential and it reduces the number of cases of malignant tumours (*Nelson et al.*, 1943; *Clayton* & *Bauman*, 1949; *Schwarz* & *Foltz*, 1957), while in 1966 the anticarcinogenic effect of selenium was reported (*Shamberger* & *Rudolp*h, 1966), but this time the total selenium content of the foodstuff was mentioned. In recent times, however, with the improved sensibility of the analytical methods, its important physiological role was established, that is, as an antioxidant along with the tocopherols, selenium participated in the metabolism, helped with the cure of certain cancers, in fact, even with the prevention, and helped maintain the healthy condition of the cell membranes. The selenium-containing glutathione peroxidase protects the unsaturated lipids by catalysing the peroxide decomposing reaction.

Foodstuffs produced in Scandinavia and in some other countries of Europe are extremely deficient in selenium. The amount of selenium getting into the human organism during daily meals (0.05-0.1 mg) is not considerable. The Hungarian soils are also extremely poor in selenium; therefore, it is not possible to satisfy the selenium needs of the organism with foodstuffs of vegetable origin. The selenium supplementation of foodstuffs is almost indispensable according to modern nutrition science. The researchers have also discovered that it is not enough to know only the total selenium content of the foodstuffs,

but it would be also necessary to know in what chemical form selenium is present in the foodstuffs, since the different chemical forms substantially differ from each other in toxicity, absorption ability and utilization in the human organism. Despite this, there is only a few number of studies in which the different selenium forms were examined.

The selenium content of the plants is determined mainly by the soil (*Terry et al.*, 2000), more exactly by the selenium content uptakeable from the soil, but not by the total selenium content. Selenide and elemental selenium ($Se^{2-}$, Se) can hardly be taken up, while the absorption of selenite and selenate ($Se^{4-}$, $Se^{6-}$) is significantly better. Practically, selenate can be fully absorbed, but a great portion of it empties with the urine before the incorporation into the protein, while only around 50% of the selenite can be absorbed, but the absorbed amount is better utilized. Beside the inorganic selenium compounds, there are seleno-amino acids or their derivatives present in the plants in substantial amounts. Foodstuffs of vegetable origin contain selenomethionine, whereas those of animal origin contain selenomethionine and selenocysteine. Selenomethionine is formed in the plants from the selenium content of the soil, and the animals can convert it to selenocystine. Selenomethionine can transform into the active form in the organism in around 90%, which is almost 100% (*Food and Nutrition Board*, 2000; *Dumont et al.*, 2004). In foodstuffs for human consumption, selenium is present mainly in the form of selenite and selenomethionine.

## 2 Materials and methods

### Materials used:

DL-SeMet and L-SeCys2 seleno-amino acid standards, DL-cysteic acid standard, *o*-phthaldialdehyde (OPA), 1-thio-β-D-glucose tetra acetate (TATG), 2-mercaptoethanol, 2-mercapto-ethane sulphonic acid, p-toluene sulphonic acid, acetonitrile, sodium acetate, acetonitrile, methanol and ethanol was purchased from Sigma Aldrich Company.

### Examinations performed:

#### I. Determination of seleno-amino acids by ion-exchange column chromatography (IEC)

The following experiments were carried out using an Ingos AAA 400 amino acid analyser:

Separation of SeMet and SeCys2 (selenocystine) standards.

Examination of the oxidation of SeMet and SeCys2.

Examination of the effect of hydrolysis performed with various hydrolysis acids.

Examination of selenic yeast nutritional supplement products.

**II. Determination of seleno-amino acids by high-performance liquid chromatography**

Determination after precolumn derivatization with OPA/2-mercaptoethanol.

Determination of SeCys2 in oxidized form after performic acid oxidation by high-performance liquid chromatography.

The derivatization and analysis were carried out with a MERCK-Hitachi HPLC containing the following modules: L-7250 programmable autosampler, L-7100 pump, L-7350 column thermostat, L-7480 fluorescence detector, AIA data conversion utility for D-7000 HPLC system manager (MERCK, Darmstadt, Germany). The separation was performed with a Superspher 60 RP-8e column or with a Purospher RP-18e 125 × 4 mm column; the temperature of the oven was 40 °C. The derivatives were detected with a fluorescence detector (ex.: 325 nm, em.: 420 nm).

# 3 Results and discussion

**I. Determination of seleno-amino acids by ion-exchange column chromatography**

*1. Separation of SeMet and SeCys2 (selenocystine)*

During the chromatographic run of the seleno-amino acid standards, using the normal chromatographic programme for protein hydrolysates, SeCys2 eluted between Val and Met, and SeMet eluted between Ile and Leu. We also attained a better separation of SeMet from Leu, which interfered under the normal conditions.

*2. Examination of the oxidation of SeMet and SeCys2*

As known, Met and Cys are determined in their stable oxidized forms: methionine-sulphone and cysteic acid. Similarly, we wanted to investigate

the behaviour of SeMet and SeCys2 against oxidizing effects. Prior to the chromatographic run, SeMet and SeCys2 were oxidized by performic acid using different temperature/duration combinations (50 °C/15 min; 30 °C/5 min and 0 °C/2 hrs). Results show that the seleno-amino acids are rather sensitive to oxidative effects, with SeCys2 found to be more sensitive than with SeMet. This latter underwent partial decomposition under the condition of 50 °C/15 min, giving – beside the peak of the rest of the starting material – two other peaks and a higher amount of ammonia on the chromatogram. Oxidation under milder conditions (30 °C/5 min and 0 °C/2 hrs, respectively) led to only a little deterioration of SeMet. Rigorous oxidation of SeCys2 (50 °C/15 min) resulted in complete decomposition with the appearance of only an ammonia peak on the chromatogram. Due to oxidation of SeCys2 under milder conditions (30 °C/5 min and 0 °C/2 hrs, respectively), a definite peak appeared at the beginning of the chromatogram (practically where normally cysteic acid elutes), which was presumably selenocysteic acid, the selenium analogue of cysteic acid.

*3. Examination of the effect of hydrolysis performed with various hydrolysis acids*

As biological samples are usually subjected to hydrolysis before an analysis, we attempted to find out how different hydrolysis agents affected the seleno-amino acids. Three hydrolysis acids were applied to the seleno-amino acid standards.

Hydrolysis with *hydrochloric acid*: this, for the proteins normally used hydrolysis method (6 M HCl, at 110 °C for 24 hrs), led to complete deterioration of both seleno-amino acids.

Hydrolysis performed with 2-*mercaptoethane sulphonic acid* (3M aqueous solution, at 110 °C for 24 hrs): this method resulted in the appearance of a peak of the hydrolysis acid at the beginning of the chromatogram in both cases, with complete deterioration of SeCys2, while SeMet remained intact.

Hydrolysis performed with *p-toluene sulphonic acid* (3M aqueous solution, at 110 °C for 24 hrs): both SeMet and SeCys2 remained practically intact, only the latter deteriorated a little.

*4. Examination of selenic yeast nutritional supplement products*

Two selenic yeast products – both containing selenium in the form of SeMet – were analysed subsequent to a hydrolysis with *p-toluene sulphonic acid*. It was experienced, however, that beside the big peaks of the amino acids present

in the samples, the presence of SeMet in small concentration could not be easily evaluated. Separation has to be further enhanced.

**II. Determination of seleno-amino acids by high-performance liquid chromatography**

The following examinations have been carried out so far:

1. *Determination after precolumn derivatization with OPA/2-mercapto ethanol*

Chromatographic run of the SeMet and SeCys2 standards was carried out after derivatization with OPA/2-mercaptoethanol. The measurement was performed on a 250×4-mm-sized C18 column, with a three-component eluent mixture (sodium acetate buffer/acetonitrile/methanol). Only SeMet appeared on the chromatogram, while SeCys2 did not form a measurable derivative with the reagents which can be measured by fluorescence detector. Detection in the UV-range was not possible due to the small sensibility.

2. *Determination of SeCys2 in oxidized form after performic acid oxidation by high-performance liquid chromatography*

Chromatographic run of the oxidized product of L-SeCys2 (30 °C/5 min and 0 °C/2 hrs) – which is presumably selenocysteic acid – was carried out along with DL-cysteic acid standard, after derivatization with OPA/TATG. L-selenocysteic acid appeared at the beginning of the chromatogram, having eluted after 4 min. The enantiomers of cysteic acid were separated and eluted later, after 17-18 min. Further investigations by HPLC are in process, which can be reported at a later stage.

## References

[1] C. C. Clayton, C. A. Bauman, Diet and azo dye tumors: Effect of diet during a period when the dye is not fed. *Cancer Research*, 1949. 9. 575–582.

[2] E. Dumont, F. Vanhaecke, R. Cornelis, Selenium speciation from food source to metabolites: a critical review. *Analytical and Bioanalytical Chemistry*, 2004. 379. 504–511.

[3] *Food and Nutrition Board*, 2000. Washington, D. C.: National Academy of Sciences Press.

[4] A. A. Nelson, O. G. Fitzhugh, H. O. Calvery, Liver Tumors Following Cirrhosis Caused by Selenium in Rats. *Cancer Research*, 1943. 3. 230–236.

[5] K. Schwarz, C. M. Foltz, Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society*, 1957. 79. 3292–3293.

[6] R. J. Shamberger, G. Rudolph, Protection against cocarcinogenesis by antioxidants. *Experientia*, 1966. 22. 116.

[7] N. Terry, A. M. Zayed, M. P. Desouza, A. S. Tarun, Selenium in higher plants. *Annual review of Plant Physiology and Plant Molecular Biology*, 2000. 51. 401–432.