

**Speciation analysis of glutathione peroxidase, selenoprotein P and selenoalbumin in human serum by tandem affinity HPLC and on-line isotope dilution ICP-MS in a healthy Greek population.**

Sophia Letsiou,<sup>1,2</sup> Tzortzis Nomikos<sup>2</sup>, Demosthenes Panagiotakos<sup>2</sup>, Christos Pitsavos<sup>3</sup>, Christodoulos Stefanadis<sup>3</sup>, Smaragdi Antonopoulou<sup>2</sup>, Spiros A. Pergantis<sup>1\*</sup>

<sup>1</sup> Department of Chemistry, University of Crete, Voutes, 71003, Greece; <sup>2</sup> Harokopeio University, El.Venizelou 70,17671 Athens, Greece; <sup>3</sup> First Cardiology Clinic, School of Medicine, University of Athens, Vas.Sofias 114, 11527 Athens, Greece; \* [spergantis@uoc.gr](mailto:spergantis@uoc.gr)

A previously published method (1,2) based on affinity chromatography (AF) HPLC (Hi-Trap Heparin and Hi-Trap Blue-Sepharose columns) hyphenated to inductively coupled plasma-(quadrupole) mass spectrometry (ICP-QMS) was further improved for the speciation analysis of selenoprotein P (SeIP), glutathione peroxidase (GPx) and selenoalbumin (SeAlb) in human serum. The proposed methodology was used to quantitatively determine selenium species in human serum samples taken from a group of healthy volunteers (N=400). The results obtained from this population, along with already published results obtained using a variety of analytical techniques, are compared and a table containing this data is presented. The latter, we consider important in order for comparisons between the various analytical techniques currently being used to be possible. To assist with such comparisons concentration units have been converted. This is important in order for direct comparison of the ICP-MS based techniques with immunoaffinity techniques to be readily made and potential differences revealed.

The mean concentration for the determined selenoproteins in the serum samples taken from a healthy Greek subpopulation (N=110) was GPx  $16.9 \pm 5.0 \mu\text{g Se/mL}$ , SeIP  $50.5 \pm 11.5 \mu\text{g Se/mL}$  and SeAlb  $10.7 \pm 4.1 \mu\text{g Se/mL}$ . According to these results 15% of Se is distributed in GPx, 45% in SeIP and 10% in SeAlb. The method accuracy for the determination of total protein-bound Se was assessed by analyzing numerous samples of a human serum certified reference material (BCR-637, 638, 639) certified for total Se content. Also comparisons with results from other publications conducting selenium speciation in BCR 637 were in good agreement.

Furthermore, we evaluated the obtained from the healthy Greek population serum protein results with anthropometric, biochemical and lifestyle characteristics of our population using biostatistical analysis (SPSS V.16). Multivariate analysis reveals a significant inverse association between SeIP and age of the participants ( $p < 0.001$ ). On the other hand sex as well as BMI of participants does not seem to influence the levels of SeIP, GPx and SeAlb in human serum.

In conclusion, this is the largest study, so far, concerning the determination of serum selenium species in an apparently healthy Greek population (This is an ongoing study with more samples continuously being analyzed). The main outcome of which was the suitability of the applied analytical method for simultaneous speciation analysis of GPx, SeIP and SeAlb in a large number of serum samples. The method proposed used proved to be robust and relatively time-efficient. Moreover, our results are rather promising since they provide baseline data for potential selenium biomarkers and will further our understanding of the function of Se in human metabolism. A clearer picture should be obtained after further work investigating relationships between serum selenium species and other biochemical, anthropometric and life style factors of the healthy volunteers.

#### References

1. Reyes LH, Marchante-Gayon JM, Garcia Alonso JI, Sanz-Medel A (2003), J Anal At Spectrom, 18:1210-1216
2. Jitaru P, Cozzi G, Gambaro A, Cescon P, Barbante C (2008), Anal Bioanal Chem 391:661-669