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Speciation Analysis of Selenoproteins from Human Serum by Affinity Chromatography Hyphenated to Inductively Coupled Plasma-Mass Spectrometry

Petru Jitaru[1,2], Marco Prete[3], Giulio Cozzi[1,3], Warren Cairns[1], Paolo Cescon[1,3] and Carlo Barbante[1,3]

[1] Institute for the Dynamics of Environmental Processes (CNR), Dorsoduro 2137, 30123 Venice, Italy, barbante@unive.it

[2] University "Al. I. Cuza", Faculty of Chemistry, Department of Inorganic and Analytical Chemistry, 11 Carol I Blvd., 700506 Iasi, Romania

[3] University of Venice Ca' Foscari, Department of Environmental Sciences, Dorsoduro 2137, 30123 Venice, Italy

Selenium is an essential element for life but is toxic at levels little above those required for health. The diverse biological effects of selenium cannot be explained by the chemical characteristics of the element itself or the few inorganic selenium compounds that living organisms are commonly exposed to. Inorganic selenium is assimilated and incorporated into a variety of organic compounds with distinct pharmacodynamic or toxic potentials, such as selenoproteins. In the last few years it has been revealed that understanding the reactions and functions of such selenium (protein) species is the key to understanding its mechanism of essentiality and toxicity, hence their speciation analysis is important.

In this study a new analytical approach based on affinity (liquid) chromatography (AF-HPLC) hyphenated to inductively coupled plasma-(quadrupole) mass spectrometry (ICP-QMS) was developed for speciation analysis of selenoprotein P (SelP), gluthatione peroxidase (GPx) and seleno-albumin (SeAlb) from human serum. Despite the analytical advantages of the on-line coupling of AF-HPLC, the determination of GPx by ICP-QMS is seriously hampered by Cl and Br, which produce serious interferences on ^{77}Se ($^{37}Cl^{40}Ar$) and ^{82}Se ($^{81}Br^{1}H$). Off-line anion exchange-solid phase extraction (AE-SPE) was carried out before the chromatographic separation in order to remove the spectral interfering elements. Efficient AF-HPLC separation of GPx, SelP and SeAlb free of interferences was obtained within a total chromatographic run time below 15 min. The repeatability in terms of relative standard deviation (RSD, %, n=10) was 7% for GPx and SelP and 3.5% for SeAlb. On-line external calibration by injecting inorganic selenium into the chromatographic system was used for the quantification of selenoproteins. Method detection limits obtained using this approach were 0.1 ng g^{-1} for GPx, 1.5 ng g^{-1} for SelP and 2.0 ng g^{-1} for SeAlb. The method accuracy in terms of total selenium was assessed by the analysis of a human serum certified reference material (BCR-639) with a certified content of total selenium. Total selenium obtained for BCR-639 with the speciation approach (GPx+SelP+SeAlb) agreed with the certified value. In addition, the method accuracy for the analysis of both control serum and BCR-639 was checked using direct ICP-QMS and ICP-sector field-MS (ICP-SFMS). In both cases quantitative recovery of selenium from BCR-639 and agreement with total selenium by speciation (GPx+SelP+SeAlb) for the control serum was obtained.