

10<sup>th</sup> World Congress on **Pharmacology**  
&6<sup>th</sup> International Conference and Exhibition on**Advances in Chromatography & HPLC Techniques**

August 02-03, 2018 | Barcelona, Spain

**HPLC estimation of antioxidants via D-phenylalanine hydroxylation and tetrahydrobiopterin during oxidative stress****Roberto Biondi**

Alessandrescu Rusescu National Institute for the Health of Mother and Child, Romania

Living organisms have developed a complex antioxidant system to counteract reactive oxygen species (ROS) and oxidative stress (OS) damage. An array of small antioxidant molecules (vitamin C, vitamin E, and flavonoid) were part of the antioxidant barrier and were evaluated using a detection system based on Fenton reaction-mediated D-phenylalanine (D-Phe) hydroxylation. This reaction in turn generated o-tyrosine, m-tyrosine and p-tyrosine that were separated by HPLC (high performance liquid chromatography) with fluorescence detectors. The addition of antioxidants competed with D-Phe on .OH attack, thus allowing to determine the .OH scavenger capacity. Using a kinetic approach, the hydroxyl radical scavenger capacity was applied to biological fluid samples but also to antioxidants as N,N dimethylthiourea. In order to define the process of ROS-mediated tetrahydrobiopterin (BH4) degradation in isolated rat hearts subjected to ischemia, a direct HPLC assay of BH4 and its pteridine derivatives was used. The lack of oxygen due to myocardial ischemia determined ROS generation and consequently caused a BH4 cardiac content decline while the dihydroxanthopterin was produced. Contrarily other researchers found a BH4 depletion with BH2 increased levels in the vessels of a range of cardiovascular disease including hypercholesterolemia, diabetes, atherosclerosis, hypertension and heart failure. HPLC estimation was based on indirect method in which BH2 and BH4 were oxidized to biopterin by iodine and then evaluated by fluorescence detector. It is debated whether the provision of OHSC (OH scavenging capacity) assay to the industries producing artificial human milk, types of tea and wine might better improve the knowledge of their antioxidant properties. Regarding to pterin biosynthetic pathway, it is discussed if the ischemia- and OS-determined BH4 alteration could result more comprehensive using a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method. This technique could be applied to amniotic fluid samples collected from pregnant women suffering from preeclampsia in whose pathogenesis the main role is due to ischemia/reperfusion- caused ROS generation.

**Biography**

Roberto Biondi completed his undergraduate degree in Medicine and Surgery from University of Perugia, Italy; pursued PhD in Biological Sciences from Pisa University, Pisa, Italy. He is currently a Visiting Professor at the Maternal-Fetal Assistance Excellence Unit, of Alessandru Rusescu National Institute for Health of Mother and Child, Bucharest, Romania. He has developed a method on the detection and scavenging of hydroxyl radical via D-phenylalanine hydroxylation in human fluids at the same institute. He has worked on the detection of tetrahydrobiopterin oxidation products in myocardial ischemia and also during chemical oxidation at both Johns Hopkins University in Baltimore, Maryland, USA and Ohio State University in Columbus, Ohio, USA.

robertobiondi42@yahoo.it