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OPTIMIZATION AND VERIFICATION OF THE METHOD FOR DETERMINATION OF ANTIOXIDANT ENZYME CATALASE IN VITRO

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The human body constantly controls the presence of pro-oxidants and antioxidants. The balance between them is closely regulated and is very important for keeping the vital cell functions. The cell is permanently exposed to attacks from different external and internal forms on reactive oxygen species. [1]The hydrogen peroxide is a compound from the biosphere and it is one of the most common reactive oxygen species. On the other side, an antioxidant enzyme catalase is well studied because of its important function in cell protection from the potentially toxic effects of hydrogen peroxide.[2]Therefore, the aim of our study was to determine the activity of erythrocyte catalase in hemolysate using modified and optimized UV spectrophotometric method of Aebi (1984). The method monitors the rate of decomposition of the substrate - hydrogen peroxide (H_2O_2) at a wavelength of 240 nm. The method was optimized in order to achieve the optimal sample dilution and optimal time point for measuring the decrease in the absorbance due to the substrate decomposition. The data obtained showed that the sample should be diluted 1:1000 to achieve the activity of catalase in the range of 0.01 KU/ml – 0.04 KU/ml. As we obtained the best correlation factor when measuring the decomposition of hydrogen peroxide 30 s after the initiation of the reaction (R2 = 0.995), we chose to measure the change in absorbance in two time points, immediately and 30 s after addition of the substrate in the reaction mixture. The performance verification of the method was assessed through the determination of the parameters linearity and reproducibility. The results proved that the method is linear in the concentration range of 0.01 KU/ml – 0.04 KU/ml. The t-test showed that the method is reproducible (there is no statistically significant difference in the results obtained in two consecutive days of measurement, p = 0,1643). With this optimized method we determined the erythrocyte catalase activity in hemolysate of 23 healthy volunteers. The blood samples were collected in the Department of Occupational Medicine in the Institute of Public Health of R. Macedonia.

Key words: catalase, acatalasemia, hydrogen peroxide, method verification, method optimization

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