Adaptation of the Phosphotungstate Method to Determine Reduced and Oxidized Vitamin C in Blood Plasma

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The phosphotungstate reagent (PTR) was used for quantitative spectrophotometric determination of physiological forms of vitamin C in blood plasma. An immediate action of PTR on the first half of the tested samples allowed to determine reduced vitamin C concentrations (I) at 700 nm. 10 mM dithiothreitol added to the second half of the samples reduced oxidized vitamin C in it – hence the total amount of this vitamin was reduced with a concentration (II) determined as above (remains of dithiothreitol were removed with N-ethylmaleimide). The difference of results (II) and (I) gave the concentration of oxidized vitamin C. The method is characterised by fault-less analytical parameters: correlation coefficients of analytical curves > 0.99, recovery factor 100.5%, variation coefficients intra- and inter-serial < 3% and < 5%, respectively, detection limit 0.05 µM.

The simplicity of the method enables an easy control of the ratio of oxidized and reduced vitamin C concentrations in blood plasma – the biomarker of the level of oxidative damage to cells.

Key words: Vitamin C – Reduced and Oxidized, Blood, Phosphotungstate Method