

ABSTRACT

α -Tocopherol is the principal membrane antioxidant in mammalian cells, although antioxidant is not the only mechanism of this vitamin. Many studies consider erythrocyte α -tocopherol levels to be more suitable to assess the tocopherol status of organism than its plasma levels. This thesis presents erythrocyte membranes α -tocopherol HPLC-UV analysis with differential ultracentrifugation and solid phase extraction sample pretreatment. Red cell samples were ultracentrifuged ($288\ 000 \times g$, 3 minutes, 4 °C) in the presence of butylated hydroxytoluene (BHT), D-mannitol, HEPES and CaCl_2 . Then α -tocopherol was extracted from erythrocyte membranes by solid phase extraction with n-hexane in the presence of ascorbic acid and tocopherol acetate internal standard. The extract was dissolved in methanol and separated on the monolithic column Chromolith Performance RP-18e (100×4.6 mm) using a 100% methanol as the mobile phase. The absorbance of α -tocopherol was measured at 295 nm wavelength.