Objective: Tocopherol is one of antioxidants in lipoprotein and cell surface, and decreases in some diseases. Therefore, the analysis of tocopherol levels in lipoprotein classes may be helpful to provide insight on the pathology of diseases in relation to oxidative stress, including diabetes and atherosclerosis. We developed a new automated method with a high-performance liquid chromatographic method (HPLC) to measure alpha- and gamma-tocopherols in HDL, LDL and VLDL fraction. Methods: The method contained diethylaminoethyl-ligand column for separation of lipoprotein classes in plasma and octadecylsilica column for separation of tocopherols in each of lipoprotein classes, and the separated tocopherols in lipoprotein were analyzed by using fluorometric detector. The analysis of one plasma sample could be completed in 50 minutes. Results: The within-assay and between-assay coefficients of variation for tocopherol levels in lipoprotein classes were less than 8 and 14 %, respectively. The correlation coefficients between tocopherol levels in lipoprotein classes estimated by the new HPLC method and the classical method, in which the tocopherol levels in ultracentrifugal lipoprotein fractions were analyzed by using reversed-phase chromatography, were larger than 0.8. Ratios of alpha-tocopherol to cholesterol in HDL, LDL and VLDL of diabetic patients were significantly lower than those of healthy subjects. Conclusions: Alpha- and gamma-tocopherol levels in HDL, LDL and VLDL could be conveniently measured by using the new developed HPLC method, suggesting that the new HPLC method awaits future clinical studies.