Aldehydes are considered potential markers for enhanced oxidative stress and have been proposed as a diagnostic measure of cancer status. Do to their volatility and activity, it is very difficult to accurately measure aldehydes in human blood. In the present work, gas chromatography/mass spectrometry (GC/MS) and solid–phase microextraction (SPME) with on–fiber derivatization was developed for determination of aldehydes in human blood. O–(2,3,4,5,6–Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) in aqueous solution was first adsorbed by a SPME fiber, and then the aldehydes in blood samples were headspace extracted by the SPME fiber and rapidly derivatized with PFBHA on the SPME fiber. Finally, the oximes formed were desorbed and detected by GC/MS in electron ionization (EI) mode. Validation of the present method was carried out, and the method was applied to quantitative analysis of the aldehydes in lung cancer blood. The results demonstrated that GC/MS and SPME with on–fiber derivatization is a simple, rapid, sensitive and solvent–free method for the determination of aldehydes in human blood.