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Qualitative and quantitative determination of biologically active low-molecular-mass thiols in human blood by reversed-phase high-performance liquid chromatography with photometry and fluorescence detection

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Abstract

The reversed-phase high-performance liquid chromatographic method employing photometry and fluorescence detection is described for the precise reproducible simultaneous measurement of total homocysteine (tHcy), cysteine (Cys), and glutathione (GSH) in human blood. Sample preparation involves conversion of disulfides to free thiols with triphenylphosphine, precipitation of proteins with trichloroacetic acid, conjugation of the thiols with monobromobimane (mBrB). The aminothiol assay is optimized by reduction and derivatization step conditions (pH, temperature and time of reactions) to obtain reliable quantitative results within the concentration range corresponding to normal and pathological levels of these thiols in human blood.

Keywords: Thiols; Aminothiols; Homocysteine; Monobromobimane; Cysteine; Glutathione