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Pre-column derivatization high-performance liquid chromatographic method for determination of cysteine, cysteinyl–glycine, homocysteine and glutathione in plasma and cell extracts

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Abstract

A sensitive high-performance liquid chromatographic method for quantification of sulphhydryl and disulfide amino acids in human plasma using ultra violet spectrophotometric detection was developed. Precolumn derivatization with 5,5'-dithio-bis-nitrobenzoic acid (DTNB) and an optional pre-derivatization reaction with dithiothreitol allowed both quantitative reduction of disulfides for measurement of total amino acid levels and the measurement of the reduced forms. A dynamic range of 500 nmol/l–750 μ mol/l allowed the major analytes of interest to be quantified in plasma without sample dilution. The assay is a sensitive and precise method for the determination of sulphhydryl and disulfide amino acids in plasma and cell extracts. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Biological compounds containing thiol groups are key reactants of intracellular reductive-oxidative

metabolic cycles, substrates for protein synthesis and enzyme cofactors. Analytical methods that quantify sulphhydryl amino acids, sulphhydryl peptides, drugs and glutathione frequently rely on high-performance liquid chromatography (HPLC) of chemical derivatives to obtain specific, sensitive and precise results. Many recent reports describe the use of HPLC with fluorescence detection of derivatives [1–8] or electrochemical detection of the sulphhydryl groups [9–12], however these detectors are not as widely available as ultra violet (UV)–visible models. To allow quantification with conventional UV-spectro-

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