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A Modified Method for the Determination of Plasma Total Homocysteine by High Performance Liquid Chromatography

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Abstract: We have modified a high-performance liquid chromatography (HPLC) procedure for homocysteine assay using 7-fluoro-2, 1, 3-benzoxadiazole-4-sulfonamide (ABD-F) pre-column derivatization. In this study, the thiol compounds were liberated from plasma proteins by reduction with 2-mercaptoethanol (BME) and derivatized with a thiol-specific fluoregenic marker, ABD-F. The derivatives were separated by reversed-phase HPLC on a Hypersil C 18 column (4.6 x 150 mm; 5 µ) using isocratic elution with 0.1 M phosphate buffer pH 6.0 containing 8% methanol and fluorescence detection. Excitation and emission wavelengths were 386 and 516 nm. The peak of homocysteine was eluted at 413 ± 25 s. Mean within-day and between-day precisions were determined to be 1.96 and 4.34% CV, respectively. The method was linear up to 100 µmol/L and proved to be sensitive with a detection limit of 1 µmol/L for homocysteine. In plasma samples from healthy adult subjects, the concentration of homocysteine was found to be higher in men than in women (11.69 ± 2.9 versus 9.45 ± 1.78 µmol/L, p = 0.002). The method is simple, sensitive, and reproducible and allows a rapid determination of total homocysteine in human plasma under routine conditions.

Key Words: Homocysteine, High performance liquid chromatography, ABD-F, Sulfamoylbenzofurazan, Mercaptoethanol

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