

HPLC Determination of Methotrexate and Its Metabolites in Blood Plasma

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Methotrexate (MTX) and its main metabolites: 7-hydroxymethotrexate and 2,4-diamino-N¹⁰-methylpteroic acid were simultaneously determined by HPLC method in human plasma sample. They were isolated from the matrix applying a solid-phase extraction procedure on a Bakerbound SPE C₁₈ column. The mean percentage recovery was 79.0, 86.9 and 85% for methotrexate, 7-hydroxymethotrexate and 2,4-diamino-N¹⁰-methylpteroic acid, respectively. Chromatographic separation of the analytes was performed on the reversed-phase C₁₈ column and isocratic elution with the phosphate buffer (pH 6.5) containing 7% of acetonitrile, 2% of N,N-dimethylformamide and 0.2% of 30% hydrogen peroxide was applied. p-Aminoacetophenone served as the internal standard. At the outlet of the column the eluent was irradiated by with UV light of 254 nm to convert the analytes to their fluorescent derivatives. The excitation and emission wavelengths were 350 and 436 nm, respectively. Quantitation limit of the investigated compounds was 0.075 µg mL⁻¹.