IMPROVED HPLC ANALYSIS WITH DOUBLE DETECTION SYSTEM OF L-DOPA, ITS METABOLITES AND CARBIDOPA IN PLASMA OF PARKINSONIAN PATIENTS UNDER L-DOPA THERAPY

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3-O-methyldopa. (OMD) is a long-lived metabolite of L-Dopa and it may be a competitive inhibitor of the enzyme dopa-decarboxylase which transform L-Dopa in the active amine dopamine. In addition 3-O-methyldopa compete with levodopa in transport through Blood-brain-barrier.

The determination of this metabolite in the blood then, correlate with the patient's clinical response, is very important in understanding L-Dopa side - effects.

Improving our previous studies we developed a simple analytical method which allows to detect OMD simultaneously at L-Dopa, and other its metabolites in plasma of Parkinsonian patients under L-Dopa therapy.

Moreover the procedure allows the simultaneous measuring of plasma Carbidopa, the dopadecarboxylase inhibitor enclosed in the formulation.

The separation is obtained with a reverse-phase column after a dual-step procedure for the prepurification of sample using a little column of CM-Sephadex for L-Dopa, Carbidopa, OMD, 3,4-dihydroxyphenylacetic acid (DOPAC) and alumina absorbtion for the catecholamines (NE, E and DA).

The detection system includes two detectors (coulometric and spectrofluorimetric) in series.

The use of two detectors increases the selectivity and the specificity of determination. At used conditions (redox mode for coulometric detector with E1=+0.25 V and E2=-0.50 V, Ex=282 nm and Em=322 nm for spectrofluorimetric detector), L-Dopa, Carbidopa, DOPAC and CA are determined with coulometric detector but for quantitative determination of OMD the spectrofluorimetric detector is used.

The method was applied to the analysis of plasma samples drawn during the ON and OFF phases from Parkinsonian patients under L-Dopa therapy.

AUTOMATED HPLC AND COLUMN SWITCHING TECHNIQUE FOR ON LINE CLEAN-UP AND ANALYSIS OF CICLOPROLOL IN HUMAN PLASMA

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An automated HPLC method allowing the direct injection of a new β_1-adrenoceptor antagonist, ciclopro lol , has been developed.

The method deals with the automatic injection of plasma sample (200 ul) on a C_{18} pre-column (40 um), clean-up of the pre-column with water in order to remove proteins and salts and transfer of the analytes to the analytical column when the quantitative chromatography takes place. While the analytical chromatography is performing, the pre-column, reset in a different path (respect to the analytical column), is flushed with different solvents in order to remove endogenous contaminants, and, so restored, is ready for another injection. The analysis is performed on a C_{18} column coupled to a fluorimetric detector. The whole process (on line clean-up and chromatography) takes about 12 minutes and is completely automatized. The detection limit of the method is about 2 $ng.ml^{-1}$ when 200 ul aliquot of plasma sample, containing suitable internal standard (propanolol), is processed.