DEVELOPMENT OF AN ANALYTICAL METHOD TO MEASURE CYCLOSPORIN A CONCENTRATIONS IN BODY FLUIDS USING HPLC

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Cyclosporin A (Sandoz, Basle, Switzerland) is a novel immunosuppressive agent which specifically inhibits T cell function after antigenic stimulation. It is a product of fungal metabolism characterised as being an extremely hydrophobic cyclic peptide consisting of 11 amino acids, with a molecular weight of 1203. The development of a suitable method of measuring amounts of the drug in body fluids has proved especially difficult. The two methods with most promise are Radio-immuno-assay and High Pressure Liquid Chromatography (H.P.L.C.). The aim of this study was to develop a rapid H.P.L.C. method of monitoring changes in Cyclosporin A concentrations in the blood of patients who were kidney or other organ recipients.

The assay was developed using two stages. Firstly Cyclosporin A was separated from as much as possible of serum protein and other constituents using C_{18} sample-preparation cartridges. The purified sample was assayed by isocratic liquid chromatography. The procedure was optimised by the analysis of the various stages involved. In order to achieve $\geqslant$ 90% recovery of Cyclosporin A and to detect concentrations as low as 100ng/ml blood, the following method was used:- 3ml methanol was added to 1ml serum to precipitate serum protein and release protein-bound drug. After standing for 16 hours at room temperature, 2ml of the clear supernatant fluid was removed, diluted by adding 1ml water, and passed through a C_{18} cartridge (Sep-Pak, Waters Associates, Ltd., Hartford, Cheshire). The cartridge was washed with 5ml water followed by 5ml 75% v/v methanol. Finally, Cyclosporin A was eluted in methanol. The first 0.5ml was discarded, as it contains no drug. The following 1ml was retained. This has been found to contain c 90% (S.D. 10.5%) of the Cyclosporin A present in the sample.

H.P.L.C. analysis was conducted in a reverse phase C_{18} column (µ Bondapack, I.D. 4mm, length 30cm, Waters Associates). The most suitable solvent was found to be Methanol : Water (95.5). At a flow rate of 1ml/minute, column temperature 60°C and detection at 220 nm (L.C.U.V., Pye Unicam Ltd., Cambridge) the retention time was 4.0 minutes (N = 5000). Cyclosporin A concentrations were measured by peak height. Although some interference with serum constituents was evident in some samples as indicated by minor changes in the peak profile, and shift from the baseline, comparison of results with RIA analysis of samples suggested good correlation between the methods.