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Full Length Research Paper

Rapid high performance liquid chromatographic determination of chlorpropamide in human plasma

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Samples were extracted with dichloromethane and the organic layer evaporated to dryness. The residue was dissolved in methanol, and 25 µl aliquot injected onto the column. Tolbutamide was used as the internal standard for chlorpropamide. The UV detector response was linear over the range 0 – 300 µg/ml, with a correlation coefficient of 0.999 and detection limit of 1.30 ng/ml. Within day and between-day assay variations was generally < 2.50%. No interference from endogenous constituent was observed. The utility of the method was demonstrated by determine chlorpropamide in samples from human volunteers following a single oral dose of 250 mg in drug interaction studies. The procedure is simple and fast, requiring small volumes of plasma.

Key words: Liquid chromatography, chlorpropamide, plasma, tolbutamide.

INTRODUCTION

A single extraction step column chromatographic assay method which is highly sensitive is described for routine monitoring aimed at correlating chlorpropamide plasma levels with clinical effect. Chlorpropamide belongs to the sulphonlurea group of hypoglycemic agents. Chlorpropamide has been in use for a number of years and it is known that wide intra-individual variation is often present after similar therapeutic doses (Hill and Crechoolo, 1978) and its therapeutic index is fairly low. Several analytical techniques have been employed in the analysis of sulphonylurea compounds from biological samples, the most frequently used being gas chromatography (Hartivig et al., 1980). The sensitivity of this method and the shorten time of analysis are advantages over older HPLC methods (Bakare et al., 1994; Chua et al., 1998). The single extraction procedure is also an advantage over a recent method (Kumasaka et al., 2005). The method developed can be used to monitor the plasma concentrations of the drugs in human subjects and elucidate further their pharmacokinetics.

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EXPERIMENTAL

Optimised HPLC conditions

The chromatograms were from an Agilent 1100 series quartenary LC pump system, equipped with ultraviolet detector, wavelength for detection was 276 nm. The stationary phase was a silica-based ultrasphere C_{18} column (5 mm, 2.0 mm × 25 cm).

The mobile phase was methanol: 0.2% acetic acid in the ratio of 3: 2; adjusted to pH 3.0 with perchloric acid. The operating temperature was ambient and the flow rate was 0.35 ml / min., with an operating pressure of 2000 psi. The sensitivity was maintained at 0.10 a.u.f.s. A short acetonitrile wash (20 min, at 1 ml/min) at the end of each day was included to remove strongly retained solutes from the column.

Reagents

All chemicals and reagents were analytical or HPLC grade. Chlorpropamide tablets were manufactured and the reference standard was donated by Neimeth International Pharm. Limited, Nigeria. Tolbutamide reference standard was donated by Alpharma (Formerly Cox Pharmaceuticals) Limited, UK. HPLC grade solvents and water were obtained by filtration through 0.45 um filters (Millipore Corp. Bedford, Mass, USA).

Standards

1 mg/ml stock solutions of reference standards (chlorpropamide

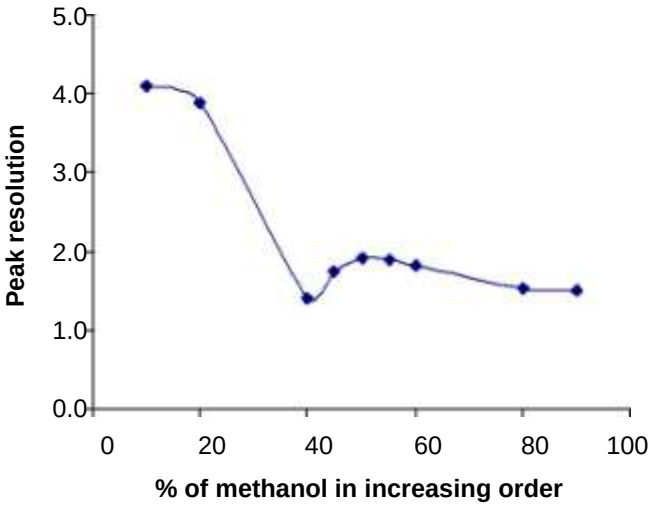

Figure 1. Effect of methanol on peak resolution during rapid high performance liquid chromatographic determination of chlorpropamide in human plasma.

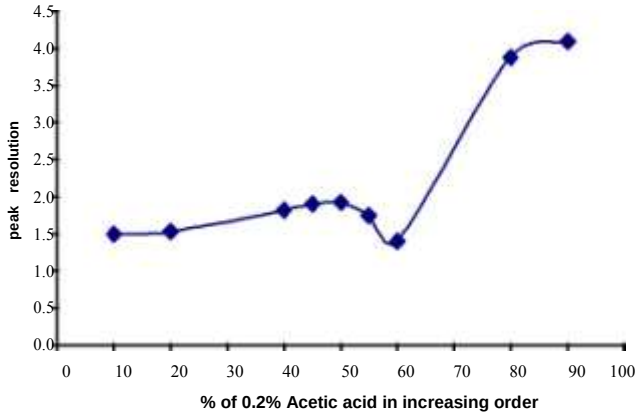

Figure 2. Effect of 0.2% acetic acid on peak resolution during rapid high performance liquid chromatographic determination of chlorpropamide in human plasma.

and tolbutamide) were prepared by dissolving 50 mg of the reference sample in 1 ml of 0.1 M sodium hydroxide and diluting to 50 ml with filtered water. These solutions were found stable up to 5 months at 4°C storage condition. Working solutions of appropriate concentrations were prepared every week by dilution of the stock solution with filtered water. The calibration curve standards were prepared by spiking drug-free human plasma with known amounts of chlorpropamide (1 – 50 ug/ml) and tolbutamide (50 ug) as internal standard.

Extraction procedure

Equal volumes (0.5 ml) of plasma, tolbutamide, and 0.1 M HCl were mixed together with 3 ml of dichloromethane and the mixture were vortexed for 15 s centrifuged at 2000 rpm for 3 min and the aqueous layer aspirated. An aliquot of dichloromethane was removed and evaporated to dryness on a water bath at 40°C. The dried residue was dissolved with 50 l of methanol, and 25 l of the solution were injected on to the column.

Calibration curve and assay

The calibration curve which was based on peak-area ratios to the concentrations of the drug, were prepared by spiking drug free serum with a standard chlopropamide (1 g/ml) to give a concentration range of 1 – 50 g/ml and 50 g of the tolbutamide was added. The level of chlopropamide was derived from these values.

Precision and sensitivity

Assay precision was determined by the analysis of drug-free human plasma spiked with known concentrations of chlopropamide. The coefficient of variation (CV) at four different concentrations of plasma chlorpropamide for 6 samples within and between-day run were determined.

RESULTS AND DISCUSSION

Extraction

Drug-free human plasma was spiked with known amount of chlorpropamide and tolbuamide (i.e), and extraction carried out with the most suitable solvent (dichloromethane) under different pH conditions 3 - 7 to compare extractability and plasma backgrounds. The average yield at pH 3 was 98.87, 87.66% at pH 4; 82.74% at pH 5. Low yield of 45.30% was obtained at pH 6 and 7.

Coefficient of variation of assay within day gave 2.1% at 1 g/ml, 1.9% at 5 g/ml, 0.96% at 30 g/ml and 0.43 at 40 g/ml. Between day assay gave 2.7 at 1 g/ml, 2.2 at 5 g/ml, 1.80 at 30 g/ml and 1.2% at 40 g/ml. The coefficient of variation for within day and between day assays at 1.30 g/ml was 0.2 and 1.9%, respectively.

Optimisation of method

The influence of mobile phase composition on the resolution (R) of peaks was assessed. Resolution (R) decreases when the methanol (Figure 1) component was increased (10 - 90%); and increases following increase in the 0.2% acetic acid (Figure 2). At different pH of the mobile phase (Figure 3), the peak area of the two drugs increases as the methanol component increases. There was a poor separation of peaks at a pH range of 3 – 5 with a corresponding decrease in resolution (R). There was an increase in resolution when the flow rate was decreased (1 – 0.35 ml/min.), and again fell bellow 0.35 ml/min (Figure 4). The best resolution of peaks and the highest detector response was obtained when the mobile phase composition (methanol: 0.2% acetic acid) was maintained at 60%: 40% respectively; and with the pH of the mixture adjusted to 3; and the flow rate kept at 0.35 ml/min. A typical chromatogram for extracted plasma is shown (Figure 5). No interference from normal endoge-

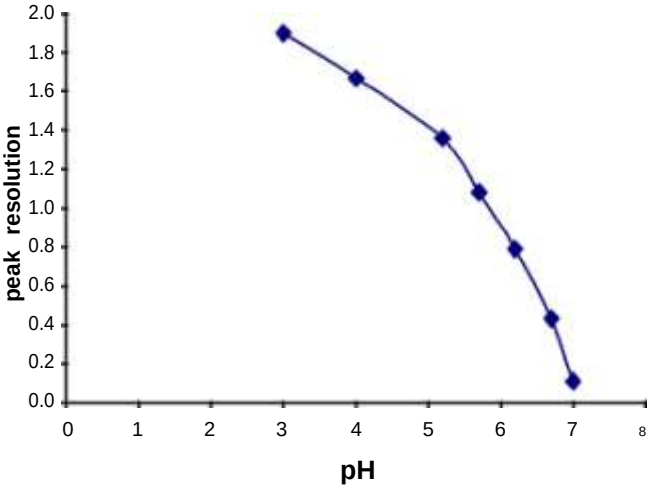

Figure 3. Effect of pH on peak resolution during rapid high performance liquid chromatographic determination of chlorpropamide in human plasma.

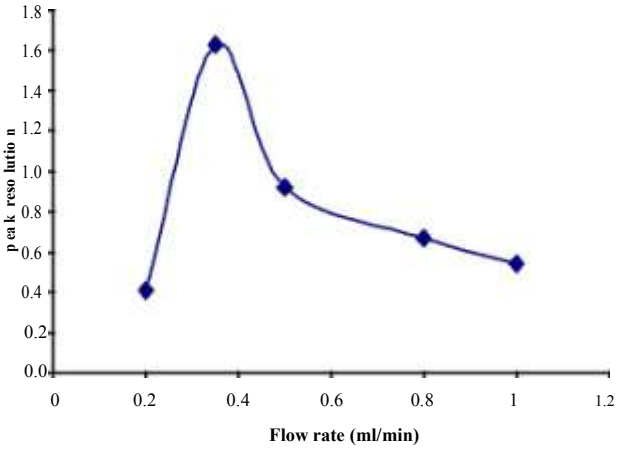

Figure 4. Effect of flow rate on peak resolution during rapid high performance liquid chromatographic determination of chlorpropamide in human plasma.

nous plasma constituents was observed. The retention times of chlorpropamide and tolbutamide (i.s) were 9 and 10.3 min, respectively.

Application of the method

The utility of the procedure described was demonstrated in the analysis of chlorpropamde in plasma samples for 3 groups (I, II and III) of human (n = 6) volunteers following pharmacokinetic drug interaction study. The mean plasma profiles of the 3 control groups following oral administration of 250 mg chlorpropamide tablet alone are shown in Figure 6. The mean plasma concentration values of chlorpropamide in all the groups (I, II and III) following

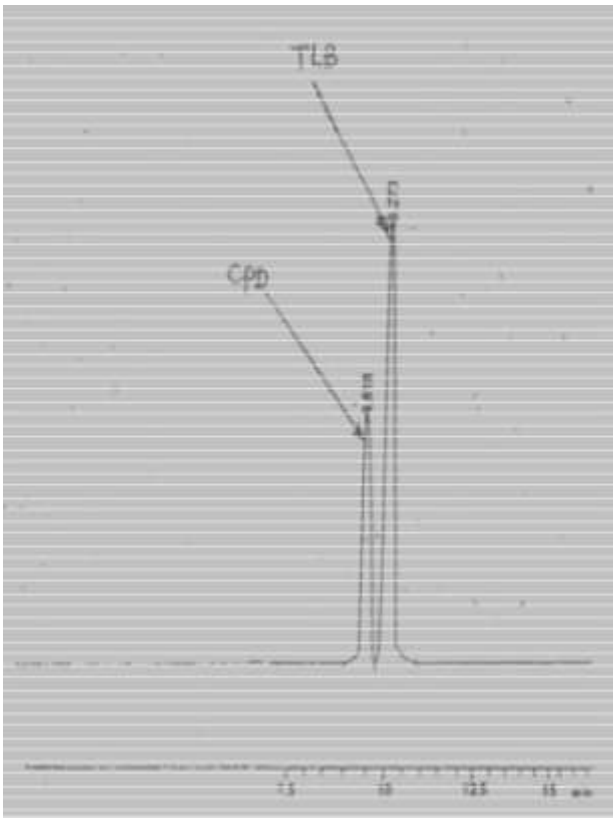

Figure 5. High performance liquid chromatograms of an extract of blank plasma containing chlorpropamide and tolbutamide (i.s).

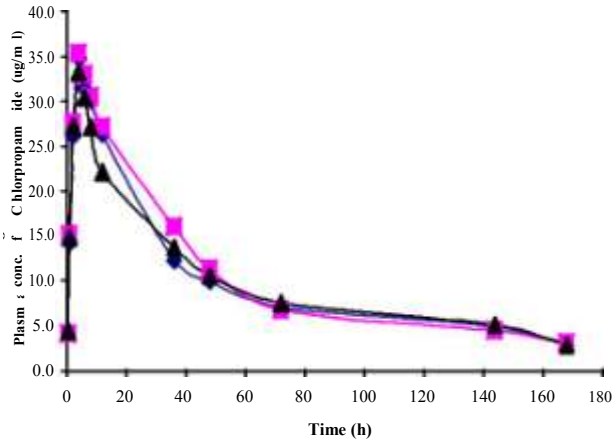

Figure 6. Mean plasma profiles of 3 (I, II, III) control groups following oral administration of 250 mg chlorpropamide tablet.

oral administration of 250 mg chlorpromide alone are in agreement with the value obtained by previous workers (Balant, 1981; Bakare et al., 1994).

This report validates the utility and suitability of this method in pharmacokinetic studies and in chlorpropa-

mide monitoring in clinical therapy. Concentration as low as 1.30 ng/ml can be detected by this procedure as against 3.5 ng/ml using GS-MS method (Chua et al., 1998).

The assay requires simple materials and it is sufficiently selective and sensitive to allow accurate and rapid therapeutic monitoring of plasma concentration of chlorpropamide in developing countries.

ACKNOWLEDGEMENTS

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