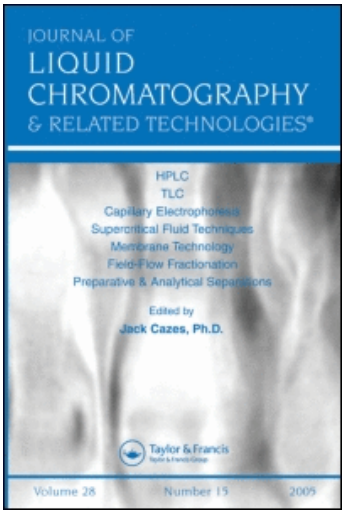

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF DESIPRAMINE IN HUMAN PLASMA USING A MICROBORE COLUMN TECHNIQUE

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ABSTRACT

A reversed-phase, isocratic HPLC method has been developed for the quantitation of desipramine in human plasma. The method involved the use of cloimpramine as an internal standard. The chromatographic separation was accomplished with a mobile phase comprising acetonitrile-aqueous solution (60:40, v/v) containing 10 mM disodium hydrogenphosphate and 80 mM sodium dodecyl sulfate adjusted to pH 2. The mobile phase was pumped at a flow rate of 0.5 ml/min. The column used was a microbore column (2 mm I.D. x 100 mm) packed with a C18 reversed-phase material (5μm ODS Hypersil). Plasma samples were extracted at basic pH with diethyl ether followed by back-extraction into 0.1 N sulfuric acid. Using UV detection at 250 nm, the lower limit of sensitivity was 10 ng/ml. The inter- and intra-assay coefficients of variation were found to be less than 10%. The assay procedure was applied to a long term oral dosing study in patients to monitor the plasma concentration of desipramine.

INTRODUCTION

Desipramine is a tricyclic antidepressant which is widely used in the treatment of patients suffering from depression. Although it has been claimed that there is no

significant correlation between plasma concentration of antidepressants and clinical response (1), it has also been suggested that tricyclic antidepressants are most effective within defined therapeutic ranges (2-4). Therapeutic drug monitoring for the most commonly used tricyclic antidepressants amitriptyline, imipramine and desipramine is becoming an acceptable practice among physicians who prescribe these drugs (5). The maximum plasma concentration of desipramine was found to be around 10-30 ng/ml following the usual therapeutic doses of the drug in man (6). A sensitive and precise assay is therefore required in order to detect the low therapeutic concentration of desipramine in plasma.

Many high-performance liquid chromatographic (HPLC) methods have already been proposed for the determination of desipramine in human plasma (7-12). Some of these published methods tested lacked the sensitivity to quantitate desipramine concentrations at the level observed in the subjects and in the patient after 24 hours post dosing. The HPLC assay described in this report has been developed and shown to be sensitive, reproducible and suitable for analysing desipramine in plasma samples in the clinical trials.

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals were of analytical grade. Desipramine hydrochloride and cloimpramine were kindly supplied by Ciba-Geigy New Zealand Ltd (Auckland, New Zealand). Sodium dodecyl sulfate (SDS) was purchased from Sigma Chemicals Co. (St Louis, MO, USA). HPLC-grade acetonitrile and diethyl ether were obtained from BDH Ltd (Poole, UK). Water was double glass distilled and MilliQ® filtered. All glassware was cleaned and silanized with 0.5% Aquasil® (Pierce, Rockford, IL, USA) before use.

Stock solution containing 100 μg/ml of desipramine was prepared in methanol. The internal standard stock solution of cloimpramine (100 μg/ml) was also prepared in methanol. The solutions were stored at -20°C until required. These solutions were found to be stable for at least 1 month. The internal standard solution used for sample preparation was 5 μg/ml cloimpramine which was prepared by dilution of the stock solution with water. Plasma standard solutions of desipramine for the calibration curves were prepared by appropriate dilution of the stock solutions with

drug-free plasma so that concentrations of 10, 25, 50, 75, 125 and 250 ng/ml were obtained. The concentration of desipramine was then determined from calibration plots of the chromatographic peak height ratios (desipramine/internal standard) versus plasma desipramine concentration.

Extraction Procedure

To 2 ml of plasma were added 100 µl of 4 M NaOH, 100 µl of the internal standard solution (cloimpramine, 5 µg/ml) and 7 ml of diethyl ether. The samples were shaken for 15 minutes on a mechanical shaker, then were centrifuged at 4°C (1500 g, 5 minutes). The ether layer was transferred to a tapered centrifuge tube containing 200 µl of 0.1 N H_2SO_4. The content was shaken for 15 minutes. After centrifugation, the organic layer was aspirated and discarded. An aliquot (70 µl) of the acidic aqueous solution was injected onto the HPLC column.

Chromatographic Conditions

The HPLC system consisted of a Model 250 Perkin Elmer LC pump (Perkin Elmer Corporation, Norwalk, CT, USA) equipped with a WISP 712 autoinjector (Waters Asso., Milford, MA, USA). A variable wavelength ultraviolet detector (Spectroflow 757, Kratos Analytical Instruments, Ramsey, NJ, USA) operating at 250 nm was used. The chromatographic response was recorded by a Hitachi D2500 integrator (Hitachi, Tokyo, Japan). A microbore HPLC column (2 mm I.D. x 100 mm) packed with a reversed-phase C18 material, 5µm ODS Hypersil (Shandon, London, UK) was used. Analysis of the samples of desipramine was performed using a mobile phase consisting of an acetonitrile-water (60:40, v/v) containing 10 mM Na_2HPO_4 and 80 mM sodium dodecyl sulfate (SDS). The pH of mobile phase was finally adjusted to 2 with orthophosphoric acid. The flow rate was 0.5 ml/min. Chromatographic separations were performed at room temperature.

RESULTS AND DISCUSSION

The mobile phase was selected on the basis of the retention behaviour of desipramine and the internal standard (cloimpramine) as a function of pairing ion

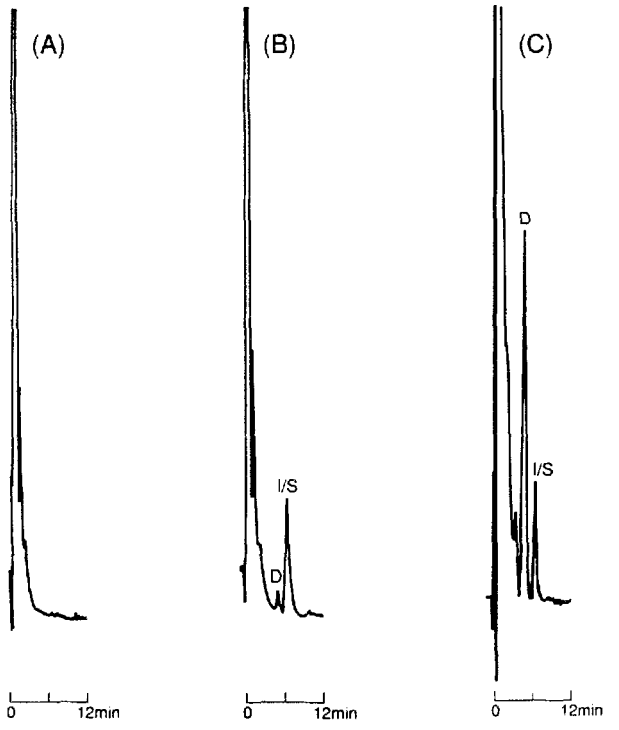

FIGURE I

Typical chromatograms of human plasma: (A) Blank plasma; (B) blank plasma containing 10 ng/ml desipramine and internal standard; and (C) plasma with 126 ng/ml desipramine from a patient 18 hours after dose of 75 mg desipramine daily. Peaks: I/S = Internal standard (cloimpramine), D = desipramine

(SDS). Chromatographic separation of desipramine and the internal standard was accomplished with a mobile phase consisting of acetonitrile-aqueous phosphate buffer mixture (60:40, v/v) containing 10 mM disodium hydrogenphosphate and 80 mM SDS adjusted to pH 2. Under the described conditions, retention times for desipramine and the internal standard (cloimpramine) were 5.0 and 6.7 minutes, respectively. Chromatograms of blank plasma, a plasma standard and a patient sample are shown in Figure 1. As can be seen, good separation of desipramine and

TABLE 1

Reproducibility of Analysis for Desipramine in Human Plasma Using the Method Described

Nominal Desipramine Concentration	n	Observed Concentration (ng/ml)[1]	C.V.[2] (%)
Within-day Reproducibility			
25 ng/ml	6	27.2 ± 2.1	7.7
250 ng/ml	6	247 ± 9.0	3.6
Between-day Reproducibility			
25 ng/ml	5	24.7 ± 2.2	8.9
250 ng/ml	5	258 ± 10.6	4.1

[1] Results are given as mean ± S.D.
[2] C.V. is a coefficient of variation

the internal standard with no interference from plasma was observed. About 10 minutes were required for each determination.

Detector response was linear to plasma desipramine concentrations of 10-250 ng/ml. The linear calibration curves for desipramine were obtained with square of the correlation coefficient (r^2) greater than 0.99. The typical linear relationship for the calibration curve can be expressed by the equation: $y = 0.0345x$, where y is the peak height ratio of the drug/the internal standard and x is the plasma desipramine concentration. The intercepts (a) in all calibration curves ($n = 7$) were found to be statistically insignificant ($p > 0.05$) and were thus not included for the calculations. The day-to-day coefficient of variation (C.V.) of the slope of the calibration curves of desipramine were 5.9% ($n = 7$).

The within-day and between-day reproducibility of the method was determined by replicate analyses ($n = 5$-6) of drug-free plasma spiked with known concentrations of desipramine. The results, expressed as mean values of the concentrations found, are given in Table 1. The coefficient of variations (C.V.) for both within-day and between-day were less than 10% illustrating the precision of the method for routine clinical purposes.

The predicted values of five 10 ng/ml plasma desipramine standards gave values of 9.8, 8.9, 9.0, 9.3 and 10.8 ng/ml. This data gives a mean and S.D. of

9.6 ± 0.8 ng/ml. The C.V. of the assay at this concentration was 8.2%, which is lower than the generally accepted C.V. of 20% for minimum quantifiable concentration (MQC). A typical chromatogram of 10 ng/ml plasma desipramine standard is shown in Figure 1 (b). Thus the MQC or the detection limit of sensitivity for this assay was assigned at 10 ng/ml.

The absolute recovery from plasma of desipramine and the internal standard (cloimpramine) was assessed by comparing the peaks of desipramine and the internal standard with those obtained from direct injection of the pure standards of the drugs of equivalent quantities. The mean recovery for desipramine (n = 4) from plasma sample was 70 ± 9% (S.D.) at 10 ng/ml and 79 ± 7% at 125 ng/ml. The absolute recovery for the internal standard was 73 ± 5% (n = 4).

Plasma samples stored at -20°C for up to two months showed no signs of decomposition and practically the same concentration values were obtained (n = 6). This suggests that desipramine is stable under these storage conditions for at least two months.

This HPLC method is now intensively used in our laboratories to analyse the plasma samples from healthy volunteers and patients receiving oral doses of desipramine in the studies to determine whether there is a relationship between debrisoquine oxidation phenotype and side effects of desipramine. The extracts of blank plasma from more than 50 healthy volunteers and patients show no endogenous compound interference. The results illustrated in Figure 1(C) indicate the method described is capable of quantitating desipramine concentrations found in plasma samples from patients receiving clinical doses of desipramine.

In summary, the method presented in this communication is simple. It is reproducible and sensitive to be applied for use both in pharmacokinetic studies and drug therapeutic monitoring in routine clinical studies.

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