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Note

Determination of diprobutine in human plasma using gas—liquid chromatography with nitrogen-selective detection

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Parkinson's disease is characterised neurologically by the death of cells of the substantia nigra and degeneration of dopaminergic neurones of the nigro-striatal tract [1]. The fundamental neurochemical defect appears to be the depletion of dopamine from the striatum [2]. In recent years drug therapy of Parkinsonism has been based on the correction of this central dopamine deficiency by the administration of L-3,4-dihydroxyphenylalanine (L-DOPA), the metabolic precursor of dopamine, or by treatment with drugs which either increase the release of dopamine from pre-synaptic nerve terminals or function as post-synaptic dopamine receptor agonists [1, 3].

Alterations in the functioning of other central neurotransmitter systems are also apparent in the pathology of Parkinson's disease. The pharmacological approach to treatment of the disease has consequently led to the assessment of a number of drugs of which the apparent mode of action is not exerted on dopaminergic function.

Diprobutine (1,1,1-tripropylmethylamine hydrochloride) is a tertiary alkyl primary amine which has been shown to possess anti-Parkinsonian activity [4, 5]. It has been extensively studied in animals and is currently undergoing clinical evaluation in man [6]. Although the mode of action is speculative, the principal effect of the drug appears to be on the noradrenergic neurones of the nigro-striatal tract in the presence of intact hypothalamic function. The effects of diprobutine on dopaminergic function are not thought to contribute significantly to its primary mode of action.

In the present communication we describe a procedure for the determination of diprobutine in plasma using gas—liquid chromatography (GLC) with nitrogen-selective detection. The method is shown to be capable of accurately measuring levels of the drug following its clinical administration in man.

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MATERIALS AND METHODS

Drug standards

Diprobutine hydrochloride and the internal standard propyl-1-isobutyl-1-butylamine hydrochloride were supplied by Labaz Pharmacy Products (U.K.) (Stockport, U.K.). A stock solution of diprobutine hydrochloride was prepared at a concentration of 0.6 *M* in glass-distilled water. This solution was used to prepare spiked plasma standards by the addition of appropriate aliquots to pooled human plasma obtained from the Regional Blood Transfusion Unit, John Radcliffe Hospital, Oxford, U.K. All batches of pooled plasma were tested prior to use to ensure the absence of any endogenous contaminating material likely to interfere with the assay. Prepared plasma standards were divided into 5-ml aliquots and stored at −20°C until required for analysis.

A solution of the internal standard, concentration 60 m*M*, was prepared in glass-distilled water. This solution was stored at room temperature and under these conditions it was found to be stable for periods of at least six months.

Reagents

All routine laboratory chemicals and solvents were of AnalaR grade and purchased from BDH (Poole, U.K.). Chlorotrimethylsilane was supplied by Applied Science Labs. (State College, PA, U.S.A.). Nitrogen (zero grade), air (zero grade) and hydrogen (CP grade) were supplied by British Oxygen Company, Special Gases Division (London, U.K.). Water was glass-distilled prior to use.

Diethyl ether was purified and rendered peroxide-free by distillation over reduced-iron powder following drying of the solvent with sodium—lead alloy. Borate buffer, pH 10, was prepared by dissolving 4.76 g of sodium borate and 18.3 ml of 0.1 *M* sodium hydroxide in a final volume of 1 l of water, to give a final pH of 10.0.

Chromatography

Gas chromatography was performed using a Model 204 gas chromatograph (Pye Unicam, Cambridge, U.K.) fitted with a nitrogen-sensitive alkali flame ionisation detector.

TABLE I

DETERMINATION OF DIPROBUTINE: SUMMARY OF GAS CHROMATOGRAPH OPERATING CONDITIONS

Injector temperature	300°C
Detector temperature	300°C
Column oven temperature	100°C (isothermal)
Nitrogen (carrier) flow-rate	25 ml/min
Hydrogen flow-rate	25 ml/min
Air flow-rate	100 ml/min
Amplifier sensitivity	$2 \cdot 10^{-12}$ to $16 \cdot 10^{-12}$ A f.s.

The analytical column (2.1 m × 2 mm I.D.) was of silanised glass and packed with 10% Carbowax 20 M + 2% potassium hydroxide on Chrom W AW, 80—100 mesh (Alltech, Arlington Heights, IL, U.S.A.). The operating conditions for the analysis of diprobutine are summarised in Table I.

The maintenance of optimum chromatographic performance required the adoption of certain routine procedures. The response to nitrogen-containing organic compounds of the alkali flame ionisation detector decreases as a function of time [7]. Additionally, detector response is affected by the composition and flow-rate of the fuel gas mixture and, in this particular instrument, the shape of the detector flame in relation to the position of the rubidium chloride alkali cation source. Prior to operating the chromatograph it was therefore necessary to optimise performance of the detector by assessment of its response to repeated injections of standard preparations of the solutes of interest under varying conditions of carrier and fuel gases flow-rates.

The operating parameters for the gas chromatograph are shown in Table I. Flow-rate values for hydrogen and air are nominal and subject to adjustment as described above. Immediately following completion of normal daily use, the analytical column was subjected to a short reconditioning cycle. The column oven temperature was increased from its working temperature to a maximum of 200°C at a rate of 2°C/min, at which temperature it was held for 60 min before being allowed to cool to 100°C. Overnight, the chromatograph was maintained under the conditions outlined in Table I.

Extraction procedure

Plasma (2 ml), contained in a 15-ml capacity conical glass centrifuge tube, was treated with 5 μl of the internal standard solution and 1 ml of borate buffer. The mixture was extracted into 5 ml of diethyl ether by vortex-mixing for 5—10 min. Following centrifugation at 2000 *g* (Minor 'S' bench centrifuge, MSE, Crawley, U.K.), the organic phase was removed and added to 2 ml of 0.1 *M* sulphuric acid contained in a separate tube. The extraction procedure was repeated as above. On this occasion, however, the organic phase was discarded. The acidic extract was treated with 100 μl of 10 *M* sodium hydroxide and further extracted with 5 ml of diethyl ether, as previously. The diethyl ether phase was then transferred to a clean glass conical centrifuge tube and reduced to dryness under a stream of nitrogen. The residue was reconstituted in 50 μl of diethyl ether; 5 μl of this solution were injected into the chromatograph.

RESULTS

Sample chromatograms obtained using the procedure described are shown in Fig. 1. Resolution (R_s) of component solutes was complete (R_s = 1.8) with a total analysis time of approximately 8 min. For routine analyses the detector amplifier was operated at attenuator settings ranging between $2 \cdot 10^{-12}$ A and $16 \cdot 10^{-12}$ A f.s. This gave a nominal limit of detection of 2 ng diprobutine base injected.

For clinical investigative work, the assay was calibrated over a concentration range from 25 to 600 nmol/l using drug-free human plasma to which had

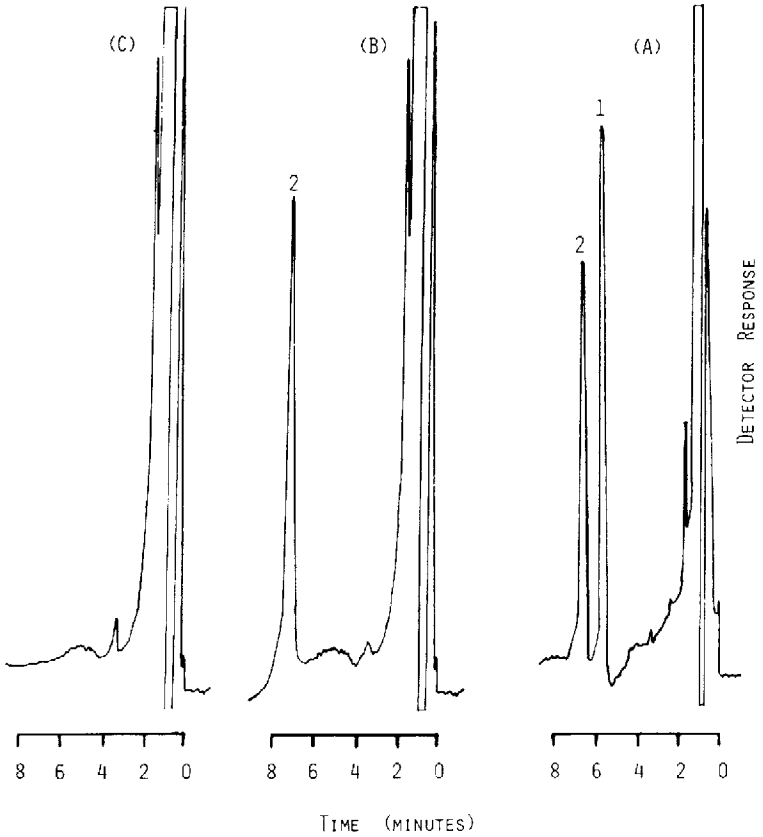

Fig. 1. Chromatograms of extracts of (A) control plasma spiked with diprobutine hydrochloride (200 nmol/l) and internal standard; (B) control plasma with internal standard only; (C) control plasma (no additions). Peaks: 1 = diprobutine; 2 = internal standard.

been added known amounts of diprobutine hydrochloride. The calibration was linear throughout this range (equation of line: $y = 0.005x + 0.04$; $r = 0.997$). Each point on the calibration curve was determined from the mean of five observations.

The precision of the extraction procedure and chromatography was evalu-

TABLE II

INTRA- AND INTER-ASSAY PRECISION OF THE GLC DETERMINATION OF DIPROBUTINE

$n = 5$.

Drug concentration (nmol/l)	Intra-assay C.V. (%)	Inter-assay C.V. (%)
25	12.5	13.9
100	9.9	5.7
200	11.0	9.3
400	6.7	10.8
600	5.9	8.6

ated by processing aliquots of pooled human plasma containing known amounts of diprobutine. Values of inter- and intra-assay variation are shown in Table II and typically ranged in both cases between 6% and 14%.

The application of the technique to the determination of diprobutine following clinical administration of the drug in man is illustrated in Figs. 2 and 3. Two healthy male volunteers (aged 26 and 32 years) were given a

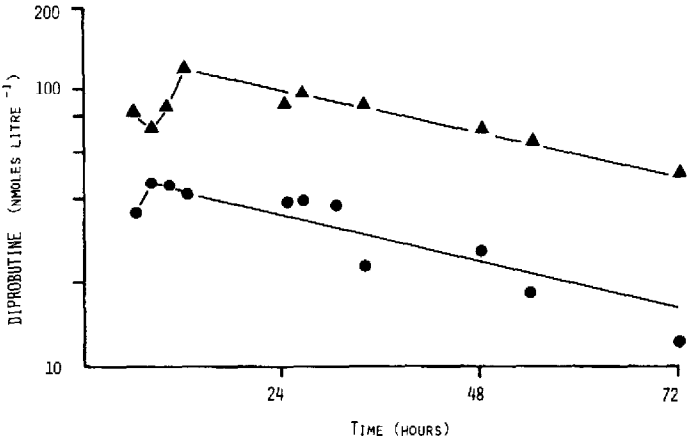

Fig. 2. Semi-logarithmic plot of plasma diprobutine concentrations in individual subjects given a single oral dose of 5 mg (•) and 15 mg (▲) diprobutine hydrochloride.

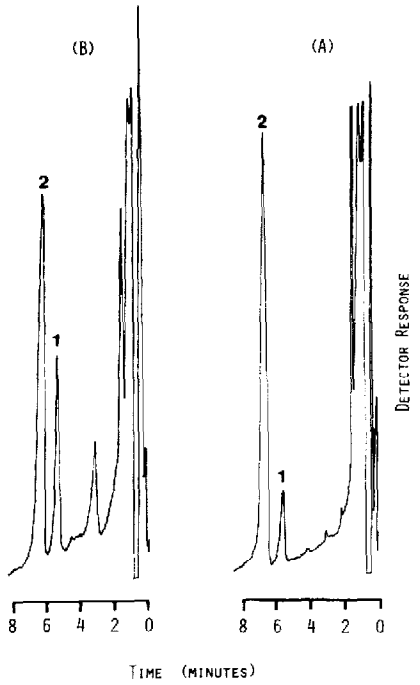

Fig. 3. Chromatograms of extracts of 12-h plasma samples taken from individuals following the administration of a single oral dose of 5 mg (A) and 15 mg (B) diprobutine hydrochloride. Peaks: 1 = diprobutine; 2 = internal standard.

single 5- and 15-mg oral dose, respectively, of the drug. Sequential plasma samples were taken for a period of 72 h following administration of the drug to obtain plasma concentration—time curves (Fig. 2) from which pharmacokinetic variables of the drug might be calculated. Fig. 3A and B shows sample chromatograms of plasma extracts from these two subjects taken 12 h after the drug had been administered. Under normal conditions plasma samples were frozen at −20°C from time of preparation to analysis. No evidence of any deterioration of stored diprobutine was observed over a period of at least eight weeks following this treatment.

Diprobutine is one of a number of drugs, demonstrated to be effective in the treatment of Parkinson's disease, whose mode of action is not primarily due to its effect on dopaminergic function. In this paper we have described a method for the determination of diprobutine in plasma using GLC with nitrogen-selective detection. The method has been shown to be sensitive, accurate and reproducible and suitable for monitoring the drug following its clinical administration in man.

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