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Short Communication

High-performance liquid chromatographic method for the determination of drotaverine in human plasma and urine

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ABSTRACT

A simple and sensitive HPLC method for the determination of drotaverine in human plasma and urine has been developed. Alkalinized plasma or urine was extracted with organic solvent and the basic components in the organic phase were back-extracted into 0.1 *M* HCl. An aliquot of the aqueous layer was injected onto the column and the eluent was monitored at 254 nm. Separation was performed on a C_{18}-column with 0.02 *M* sodium dihydrogen phosphate–methanol (30:70, v/v) containing perchlorate ion at pH 3.2 as mobile phase. Drotaverine was well resolved from the plasma constituents and internal standard. An excellent linearity was observed between peak-height ratios and plasma concentrations and the intra- and inter-assay coefficients of variation were always $< 10\%$. The lowest limit of detection (signal-to-noise ratio 3:1) was 6 ng/ml. The method is suitable for therapeutic monitoring and pharmacokinetic studies of drotaverine in humans as well as in animal models.

INTRODUCTION

Drotaverine (I, Fig. 1), a benzylisoquinoline derivative, is an analogue of papaverine with excellent smooth muscle relaxant properties. It is available as the HCl (No-Spa, Chinoin, Hungary) and theophylline-7-acetic acid (Depogen, Chinoin, Hungary) salts. It is rapidly absorbed and readily metabolised in the liver by O-deethylation to the mono- and di-phenolic compounds and their corresponding glucuronic acid derivatives. Excretion occurs through the kidney although the metabolites are readily excreted into the bile [1,2].

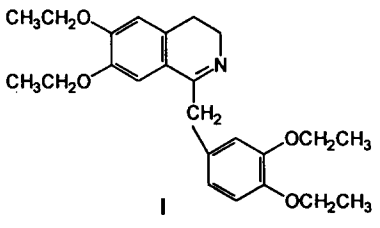

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Fig. 1. Structure of drotaverine.

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Though more effective as an antispasmodic than papaverine, the side effects of drotaverine are similar. This incidence of side effects is dose dependent [3] and a useful pharmacological correlation can be made by measuring plasma concentrations of the drug. However, there is relatively little information on the measurement of blood levels of drotaverine following administration to humans.

Total radioactivity measurements, using ^{14}C-labelled drotaverine have been used to study the pharmacokinetics of the drug in mice [4], rats [2,5] and humans [6,7]. This method lacks selectivity and is not readily applicable to bioavailability and pharmacokinetic studies. Although, two-dimensional densitometry was used to quantitate the metabolites of ^{14}C-labelled drotaverine in rats [1], the time necessary for completion of the chromatographic runs with analysis of a large number of samples may be a limiting factor.

High-performance liquid chromatography (HPLC) offers rapidity, selectivity and sensitivity and is increasingly becoming the method of choice for the analysis of drugs in biological fluids. The only HPLC method [8] reported for the quantitation of drotaverine in dog and rat plasma requires a lengthy extraction process and lacks sensitivity required for pharmacokinetic studies in man.

The present report describes a rapid, simple and highly sensitive HPLC method for the determination of drotaverine in human plasma and urine.

EXPERIMENTAL

Reagents and materials

Drotaverine-HCl and No-Spa tablets were gifts from Imarsel Chemical Company (Lagos, Nigeria). Imipramine (HCl salt), used as the internal standard (I.S.), was purchased from BDH (Poole, Dorset, England). Methanol and diethyl ether (Koch-Light, Suffolk, England) were analytical reagent grade and were re-distilled before use. Sodium dihydrogen phosphate and perchloric acid (BDH) were used without further purification.

Stock solutions containing 100 μg/ml of drotaverine and imipramine (I.S.) respectively were prepared in distilled water. The required working standards were prepared daily from the stock solutions.

Chromatographic conditions

A Varian Model 500 Liquid Chromatograph (Varian AG, CH-6300 Zug, Switzerland) fitted with a fixed wavelength UV detector (254 nm) and a 10-μl Varian manual loop valve injector was used for the analysis. The column was a reversed-phase C_{18} 10-μm Micropak MCH-10 column (300 × 4.0 mm I.D.) (Waters Associates, Milford, MA, USA). The detector output was connected to a Perkin-Elmer Model 56 recorder (Perkin-Elmer, Beaconsfield, Bucks, England). A mobile phase of 0.02 *M* sodium dihydrogen phosphate–methanol (30:70, v/v) containing 70 mmol/l perchloric acid was pumped through the column at a flow-rate of 1.2 ml/min. The pH of the mobile phase was 3.2 and the chromatogram was run at ambient temperature.

Extraction

To 1 ml of plasma or urine (1:5 dilution with water) placed in a 10-ml tapered extraction tube was added 1 ml of 2 *M* NaOH, 25 μl of I.S. solution (10 μg/ml) and 4 ml of diethyl ether. After vortex-mixing for 1 min followed by centrifugation at 2000 *g* for 10 min, the ether layer was transferred in another tapered-end tube. HCl (100 μl, 0.1 *M*) was added to the ether layer, and the mixture was vortex-mixed for 1 min and centrifuged for a further 5 min. A 10-μl aliquot of the acidic aqueous extract was injected onto the HPLC.

Calibration curves

Calibration curves based on the peak-height ratios of drotaverine to that of I.S. were prepared by spiking drug-free plasma and urine with standard drotaverine solutions (5 and 20 μg/ml) to give a concentration range of 0.025–1.0 μg/ml. The solutions were taken through the procedure described above.

Linear regression analysis of these ratios *versus* the known drotaverine concentrations gave a slope and intercept from which the equation of a straight line was obtained. Drotaverine concentrations in subject samples were obtained by

substitution of the experimentally determined peak-height ratios into the equation for the standard curve prepared for the analysis of every set of samples.

Plasma level studies

To demonstrate the applicability of the procedure to measurement of drotaverine levels in pharmacokinetic studies, 80 mg of drotaverine-HCl (No-Spa) was administered orally, after an overnight fast, to two healthy male volunteers. Blood samples were collected prior to dosing and at various times up to 30 h after dosing. The blood samples were centrifuged and the plasma analysed as described above or stored at −20°C. Total voided urine between 0 and 6 h after drug administration was also collected.

Precision and recovery studies

Validation of the method was performed by assaying six replicate samples of blank plasma and urine spiked with drotaverine standard solutions to give predetermined concentrations. To each sample was added 25 μl of I.S. (10 μg/ml) before taking it through the analytical procedure previously described. Difference between the estimated and mean added concentrations was taken as the accuracy of the method while the precision was estimated by calculating intra- and inter-day coefficients of variation (C.V.). The absolute recovery was determined by comparing the peak-height ratios of extracted plasma and urine spiked with drotaverine standards with those obtained from direct injections of appropriate standard drug solutions.

RESULTS AND DISCUSSION

Shown in Fig. 2 are typical chromatograms of a blank plasma and a real sample obtained from

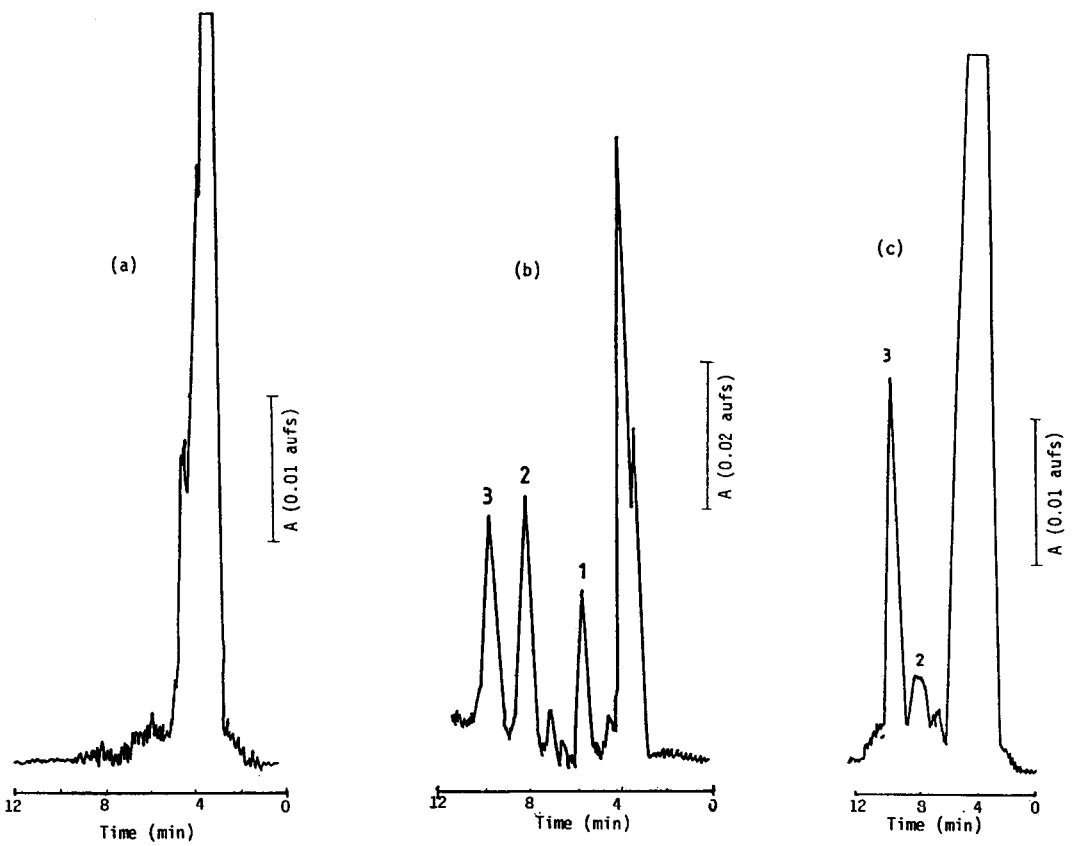

Fig. 2. Chromatograms of the extract of (a) blank human plasma, (b) plasma obtained from a volunteer 3 h after oral administration of 80 mg drotaverine HCl (concentration of drotaverine = 320 ng/ml), and (c) 0–6 h urine sample from the same volunteer (concentration of drotaverine = 99 ng/ml). Peaks: 1 = unidentified metabolite, 2 = drotaverine, 3 = internal standard.

TABLE I

PRECISION AND RECOVERY OF ANALYTICAL METHOD ($n = 6$)

Drotaverine concentration (ng/ml)	Recovery (mean ± S.D.) (%)	Precision (C.V.) (%)	
		Intra-assay	Inter-assay
Plasma			
50	93.0 ± 11.2	8.3	7.1
500	85.0 ± 7.8	7.8	9.2
Urine			
50	94.3 ± 8.6	7.6	8.2
500	89.8 ± 6.3	7.9	9.1

a subject after administration of 80 mg of drotaverine HCl. Drotaverine and the I.S. were well resolved and the retention times were 8.0 and 10.0 min respectively. No interfering peaks were observed in the chromatogram of the extracted plasma constituents. A third peak, absent in blank and spiked plasma appeared at 6.0 min. This peak is due to an unidentified metabolite of the drug.

There was a linear relationship between the ratios of the peak heights of drotaverine to that of the I.S. and the corresponding concentrations of drotaverine in the examined concentration range of 0.025–1.0 μg/ml. The regression equation typical for calibration curves were $y = 0.0028x - 0.002$ with a coefficient of correlation (r^2) of 0.998 ($n = 6$) for plasma and $y = 0.0029x - 0.0078$ ($r^2 = 0.997$) for urine, where x = drotaverine concentration in ng/ml and y = corresponding peak-height ratios. The limit of detection was 6 ng/ml (signal-to-noise ratio 3:1).

The method was found to be reproducible as indicated by the low coefficient of variation (C.V.) obtained while absolute recoveries of drotaverine, calculated from six replicate determinations, were nearly quantitative ranging from 85 to 93% in the two biological fluids at concentrations of 50 and 500 ng/ml (Table I).

Plasma concentration–time courses of drotaverine in two healthy subjects after a single oral dose of 80 mg (2 × 40 mg tablets) are depicted in Fig. 3. Peak plasma concentrations were 136 and 320 ng/ml respectively at 2 h while plasma concentrations of the drug varied widely in the two subjects. Elimination of the parent

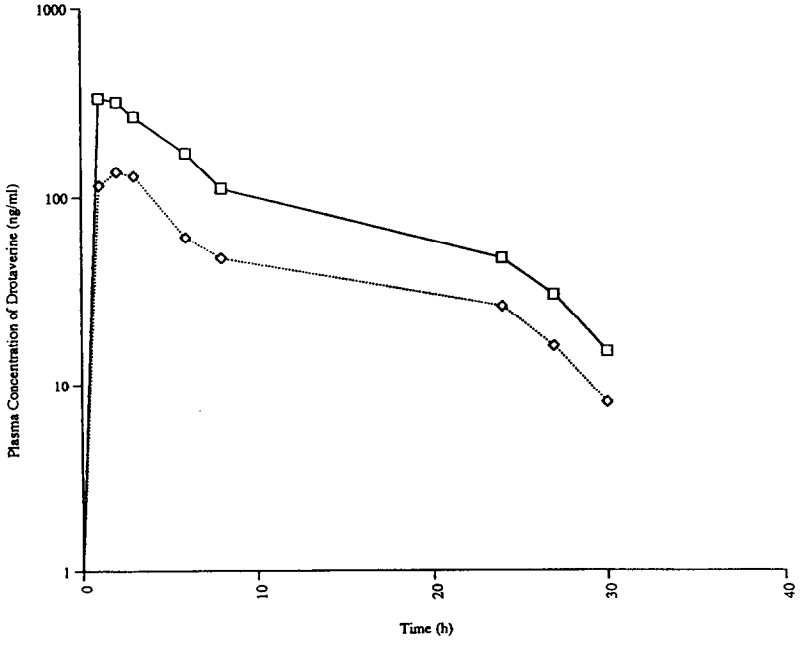

Fig. 3. Time course of plasma concentration of drotaverine in two human subjects given a single oral dose of 80 mg of drotaverine hydrochloride. (——) Subject A, (· · · · ·) subject B.

drug in urine was very low, the total amounts recovered 6 h after drug administration in the two subjects were 59.4 and 66.5 μg respectively. This finding is in agreement with previous studies that drotaverine is rapidly and extensively metabolised by the liver [1,7].

In summary, a rapid, sensitive HPLC method has been described for the determination of drotaverine in human plasma and urine. This analytical procedure is reproducible and less cumbersome than previously described methods. It is readily applicable to routine analysis of plasma samples in bioavailability and pharmacokinetic studies in humans and also for therapeutic monitoring.

After this manuscript was submitted for publication we became aware of the recent report by Lalla *et al.* [9] on their HPLC procedure for the analysis of drotaverine in human plasma. While the method is more or less similar in simplicity and reproducibility, one advantage of the method we have developed is that it does not require plasma extracts to be evaporated to dryness during the work-up procedure. The report by Lalla *et al.* [9] also contains no pharmacokinetic data.

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