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Note

Column liquid chromatographic determination of prajmalium in plasma and urine by direct sample injection

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Prajmalium is an antiarrhythmic drug chemically related to ajmaline (N-propylajmaline). Despite its quaternary structure this drug exhibits a lipophilicity greater than that of ajmaline, owing to the existence of a non-polar tautomeric form [1]. Prajmalium is therefore claimed to have a good oral bioavailability and has been successfully employed in the long-term treatment of various types of arrhythmia [2–5]. Its pharmacokinetic features are not thoroughly known and the therapeutic range of its plasma levels is still to be fully defined. A number of analytical procedures have been described for the quantitation of prajmalium in biological fluids [6–9], including radiolabelling coupled with thin-layer chromatography [6], and solvent extraction followed by direct fluorimetric detection [7] or by gas chromatography (GC) [8] or high-performance liquid chromatography (HPLC) [9]. All these methods, however, are non-specific [7] or expensive [6] or time-consuming [6,8,9].

In 1981, Roth et al. [10] described a fully automated HPLC system that made it possible to assay some drugs by direct on-column injection of plasma and urine. The equipment is time-saving but rather complex; it consists of one autosampler, two pneumatically driven electronically controlled valves, two pumps, and other devices. The same chromatographic principle was applied by us to develop a simple, non-automated apparatus for prajmalium determination in plasma and urine.

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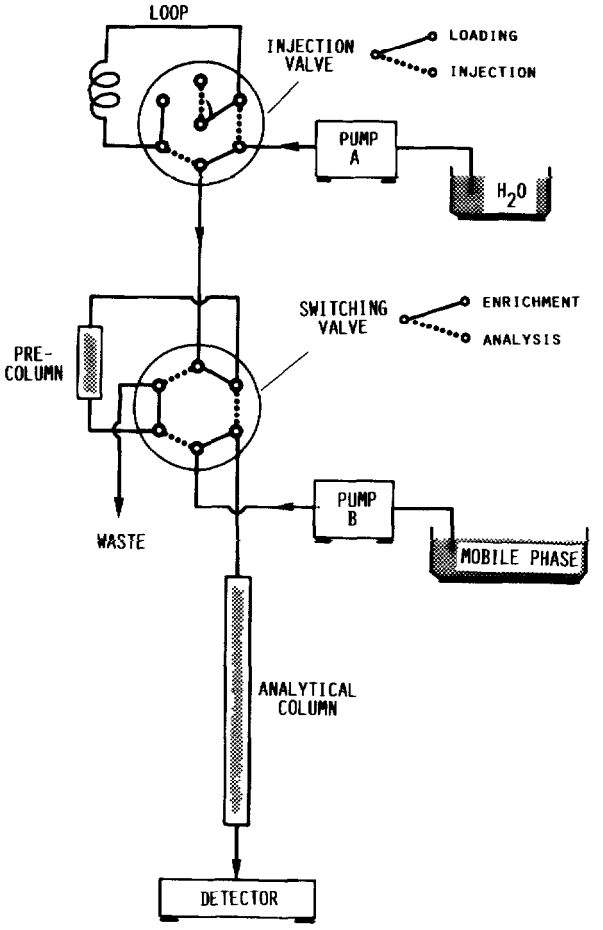

Fig. 1. Components and connections of the apparatus.

EXPERIMENTAL

Chemicals

Prajmalium bitartrate was obtained from Byk-Gulden Italia (Milan, Italy) and the injectable ampoules for the pharmacokinetic study (Neo-Gilurytmal®) were supplied by Giulini Pharma (Hannover, F.R.G.). Acetonitrile was HPLC grade and the other reagents used were reagent grade.

Apparatus and technique

Fig. 1 shows the various components of the apparatus. It consists of two pumps: the first pump (pump A: Bio-Rad Model 1330) is connected to an injection valve (Rheodyne Model 7125) equipped with a 2-ml loop, and the second (pump B: Perkin-Elmer Series 2) to a switching valve (Rheodyne Model 7000). After injection the pump A mobile phase (water) carries the sample (0.5 ml of plasma or 0.1 ml of urine) to the switching valve. When the switching valve is in the

"enrichment" position the sample passes through a reversed-phase pre-column where the drug is adsorbed on hydrocarbon groups while hydrophilic components of the sample are run out. At the same time pump B perfuses an analytical column with an appropriate mobile phase (see below). Then, 3 min after injection, the switching valve is turned to the "analysis" position so that (1) the pump A mobile phase is shunted outside the system and (2) the pump B mobile phase flows through the pre-column in the "backflush mode" and the adsorbed compounds are eluted onto the analytical column. The eluent is then analysed by a fluorescence detector (Perkin-Elmer 650s; excitation wavelength, 280 nm, emission wavelength, 360 nm; slit width, 10 nm; range, $\times 10$).

When the analysis has been performed the switching valve is turned back to the enrichment position for 3 min, thus allowing the pre-column to re-equilibrate in the water phase. To save time the re-equilibration can also be started 3 min before the chromatogram is completed. The whole process can then be repeated.

Before injection, plasma (obtained by treating blood with EDTA) and urine are filtered (0.22-μm Millex-GS filter, Millipore) in order to remove any solid particles that could clog the pre-column head frit. In spite of this, the working pressure of pump A showed a tendency to rise from the initial value of ca. 3.5 MPa; nevertheless, no qualitative or quantitative change was observed in the shape of the chromatogram. As a precautionary measure, the upper part of the pre-column stationary phase was replaced and the head frit cleaned as soon as the pressure had reached 7 MPa, normally after not less than 50 injections.

Mobile phases

Two different mobile phases were employed. (1) Pump A (enrichment/purge): tap water purified by a Milli-Q reagent water system (Millipore); flow-rate, 2 ml/min. (2) Pump B (elution/chromatography): acetonitrile–1% ammonium acetate buffer pH 4.7 (50:50); flow-rate, 0.9 ml/min.

Columns

The pre-column was obtained by cutting a 30-mm segment of an analytical column (4.6 mm I.D.) and was packed with Perisorb RP-18 (30–40 μm particle size). A 125×4 mm I.D. LichroCART RP-8 (3 μm particle size) was employed as the analytical column.

RESULTS AND DISCUSSION

The chromatograms obtained from blank and authentic samples show (Fig. 2) that neither 0.5 ml of plasma nor 0.1 ml of urine produces endogenous peaks interfering with the analysis of the drug. Other authors [9], using an ion-pair liquid chromatography technique, achieved complete separation of the two stereoisomeric forms of prajmalium; the single peak observed by us suggests that under our experimental conditions even partial isomer separation cannot be accomplished.

Although the retention time of prajmalium is 6.5 min, some endogenous peaks from plasma samples have longer retention times and prolong the analysis. Thus,

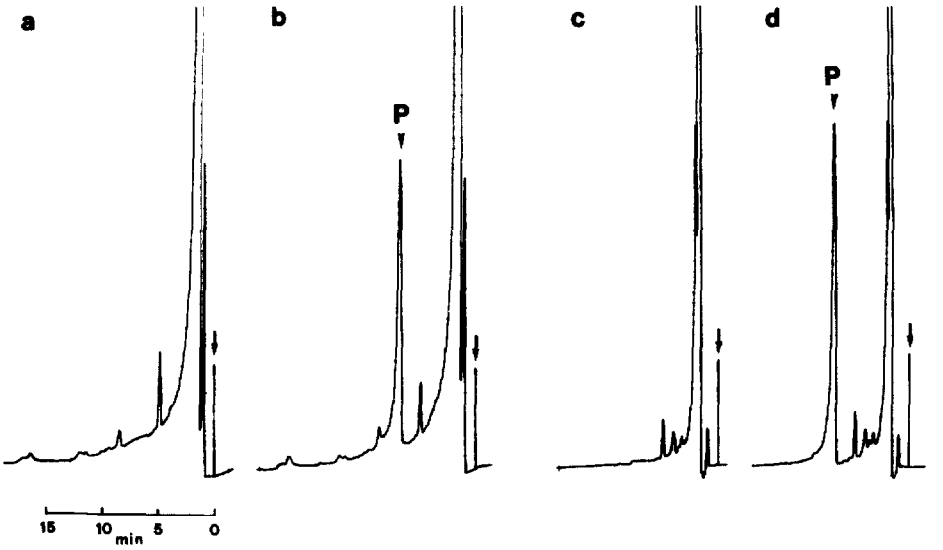

Fig. 2. Chromatograms obtained from (a) blank plasma (0.5 ml), (b) plasma containing 176 ng/ml prajmalium (0.5 ml), (c) blank urine (0.1 ml), (d) urine containing 968 ng/ml prajmalium (0.1 ml). Peak P = prajmalium. The arrows indicate the injection of the samples.

the entire cycle (enrichment–chromatography–re-equilibration) can be carried out in ca. 20 min. Variations in sex and age do not by themselves introduce quantitative changes in the chromatogram shape, but the height of the endogenous peaks may vary among subjects. When the linearity of the peak height–concentration relationship was tested over the 50–800 μg/l range ($n=6$) the coefficient of correlation was always greater than 0.99 and the line passed through the origin. It therefore seems that measurement of the peak area could not appreciably improve the precision of the method. The coefficient of variation

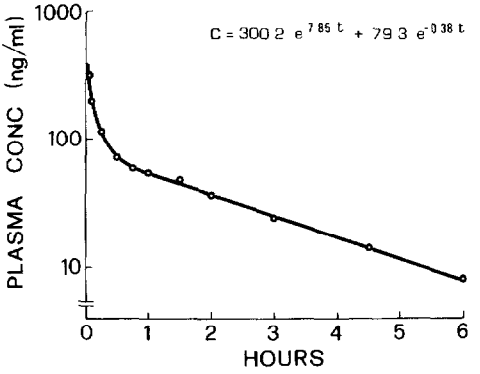

Fig. 3. Time course of prajmalium plasma concentrations after i.v. administration of 10 mg. The following kinetic parameters were calculated: central compartment volume of distribution (V_d) = 26 l; total V_d = 127 l; total plasma clearance = 676 ml/min; renal clearance = 58 ml/min; distribution plasma half-life ($T_{\frac{1}{2}}$) = 5.3 min; elimination $T_{\frac{1}{2}}$ = 1.8 h. The data were fitted to a two-compartment open model.

(C.V.) was calculated at 50 μg/l ($n=8$), at 200 μg/l ($n=8$) and at 500 μg/l ($n=8$); the results were 4.9, 4.4 and 3.8%, respectively.

To assess the day-to-day reproducibility of the method, five plasma samples spiked with 50, 100, 200, 400 and 800 μg/l were analysed and frozen; after seven days they were thawed and re-analysed. The results obtained on the two separate occasions differed by 0.8–8.2%, depending on the drug level tested. The mean drug recovery from the plasma compared with that from spiked aqueous solutions was 89% at 50 μg/l ($n=8$) and 96% at 500 μg/l ($n=8$). In urine the C.V. and the mean recovery were calculated at 500 and 2000 μg/l, namely at the limits of the range of concentrations expected in urine collected within 6 h after a 10-mg intravenous (i.v.) dose. The C.V. was 3.3% at 500 μg/l and 1.9% at 2000 μg/l, and the mean recovery was virtually 100% at both concentrations.

The use of an internal standard is not strictly required, provided that the volume injected is adjusted with care; in fact, results were not improved by the addition of verapamil hydrochloride (1 mg/l) as internal standard. The minimum detectable level of the drug, measured as the prajmalium concentration giving a signal-to-noise ratio of 2.5:1, was ca. 5 μg/l for 0.5 ml of plasma and 25 μg/l for 0.1 ml of urine.

The injection of blank plasma or urine after repeated injections of samples spiked with prajmalium did not produce "memory effects". As an example of pharmacokinetic application, Fig. 3 depicts the time course of prajmalium plasma levels in one patient affected by ventricular arrhythmias after the i.v. administration of a 10-mg dose. The values of the main kinetic parameters calculated are shown in the Fig. 3 legend.

In conclusion, the method described in this paper proved to be sensitive, reproducible and specific and seems suitable for pharmacokinetic studies and routine drug monitoring. Moreover, the equipment required is relatively simple to set up by adding a pump and a switching valve to a pre-existing chromatographic system.

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