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Note

High-performance liquid chromatographic analysis of a new antiarrhythmic drug, pirmenol, in biological fluids

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Pirmenol hydrochloride (Fig. 1) is a new antiarrhythmic drug [1, 2] that is currently undergoing initial human studies in our institution. In general,

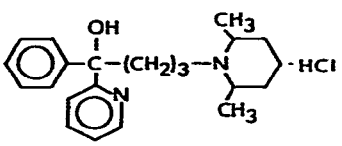

Fig. 1. Structure of pirmenol hydrochloride.

such compounds have a narrow therapeutic index so that early definition of the therapeutic range of plasma concentrations and pharmacokinetics of the drug are of great importance. Initial animal studies [1, 2] reported effective plasma concentrations of the order of 1—3 μg/ml. The method used was a fluorometric dye technique developed by the Drug Metabolism Group at Warner-Lambert/Parke Davis, but no details of the procedure were given.

The present paper describes a rapid, sensitive, selective and accurate high-performance liquid chromatographic (HPLC) method for the measurement of pirmenol in blood plasma and urine.

EXPERIMENTAL

Reagents and materials

Pirmenol (*cis*-(±)-α-[3-(2,6-dimethyl-1-piperidinyl)propyl]-α-phenyl-2-pyridinemethanol monohydrochloride) was kindly supplied by Warner-Lambert/Parke Davis (Ann Arbor, MI, U.S.A.) and the internal standard, disopyramide

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[4-diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide] by Searle Labs. (Chicago, IL, U.S.A.). Stock solutions of pirmenol in methanol and disopyramide in 0.01 *M* phosphoric acid were stored at 4°C for 4 months without detectable decomposition. Ammonium dihydrogen phosphate and triethylamine were purchased from Aldrich Chemical (Milwaukee, WI, U.S.A.). Acetonitrile was of HPLC grade obtained from MC/B-EM (LiChrosolv; Cincinnati, OH, U.S.A.). All other solvents and reagents were of reagent grade.

Sample preparation

Whole blood or plasma (0.5 ml) is placed in a 15-ml capacity culture tube, fitted with a PTFE-lined screw cap and the internal standard (50 μl containing 1 μg of disopyramide), 100 μl of 1 *N* sodium hydroxide and 4 ml of diethyl ether were added. The samples are then immediately extracted using a Labquake automatic shaker for 10 min, followed by centrifugation at 1000 *g* for 5 min, to separate organic and aqueous phases. The lower, aqueous phase is frozen by immersion of the tube in a dry ice—acetone bath and the organic phase transferred to another tube with an elongated cone of about 100 μl capacity. Then 80 μl of 0.1 *N* phosphoric acid are added and the mixture agitated in a Vortex mixer for 40 sec. After a brief centrifugation, 50 μl are sampled from the cone and injected into the chromatograph.

In the case of urine, 500 μl are mixed with 2 ml of 0.1 *N* sodium carbonate (pH 11.23) and 500-μl aliquots extracted in the same way as blood and plasma, except that sodium hydroxide is not added.

Chromatography

The HPLC system consists of a solvent delivery system (Constametric III pump, LDC System, Riviera Beach, FL, U.S.A.) and a 50-μl fixed volume loop injector (Rheodyne 7125, Berkeley, CA, U.S.A.). The analytic column is a reversed-phase Dupont's Zorbax™ TMS (250 × 4.6 mm I.D., particle size 6 μm). The mobile phase is a mixture of acetonitrile—0.05 *M* ammonium dihydrogen phosphate—triethylamine (15:85:0.5, v/v), the pH being adjusted to 2.6 with 1.0 *M* phosphoric acid. The solvent flow-rate is 1.1 ml/min with a column inlet pressure of 1800 p.s.i. The eluate is monitored continuously for absorbance changes at 262 nm using a Spectro-Monitor III (LDC Liquid Chromatogram) and the chromatograms displayed on a Linear Instruments Model 858 dual pen recorder (Irvine, CA, U.S.A.).

Calibration and accuracy

Calibration curves were constructed for each series of unknowns by adding standards of 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 3.0 μg of pirmenol and the internal standard (1 μg disopyramide) to control samples of blood and plasma. In the case of urine the standards were 5, 10, 15, 20, 25, 30 and 35 μg of pirmenol. The peak height ratio (PHR) of pirmenol to the internal standards was divided by the amount of pirmenol to derive the normalised PHR. The normalised PHR was used to calculate the amount of pirmenol in the samples and the standard deviation of the normalised PHR used to estimate the accuracy of the method.

The reproducibility of the method was examined by analysing eight replicate plasma samples containing 0.1—2.0 μg/ml pirmenol.

Application of the method

A male patient volunteer with premature ventricular ectopic beats received a 30-min infusion of 150 mg of pirmenol. Samples of venous blood were collected at various intervals for 12 h into heparinised (10 units per ml blood) glass tubes. Aliquots (500 μl) of whole blood were transferred to extraction tubes and stored at −70°C until analysis. The remaining blood was centrifuged to obtain plasma which was also stored at −70°C until analysis. Urine was collected for 24 h and frozen until assay.

RESULTS AND DISCUSSION

The present HPLC method for pirmenol analysis involves an ether extraction after sample alkalinization, followed by back extraction into phosphoric acid and reversed-phase chromatography using a Zorbax TMS column and UV detection. Diethyl ether was chosen as the extraction solvent after an initial comparison with ethyl acetate which resulted in lower and more variable recoveries and methylene chloride which, though efficient, extracted interfering peaks. Back extraction was chosen over evaporation of the organic phase over nitrogen because more reproducible results were obtained. The extraction procedure gave recoveries of 70 ± 1.5, 47 ± 2.9, and 91 ± 3.8% from four samples of plasma, blood and urine, respectively. The Zorbax TMS column was chosen after comparison with a Waters Assoc μBondapak C_{18} column which proved less satisfactory because of tailing.

Chromatograms from control plasma and plasma from the patient after pirmenol administration are shown in Fig. 2. Although our experience is, as yet, limited we have analysed control plasma from patients who were receiving quinidine, digoxin, diazepam, ibuprofen, propranolol, hydrochlorothiazide and indomethacin and found no interfering peaks. However, the chosen internal standard, disopyramide, is also an antiarrhythmic drug, so that its use would not be appropriate were patients to receive this particular drug combination. During the initial investigations of pirmenol, other antiarrhythmic drugs will be avoided, but we are currently exploring pirmenol analogues as potential internal standards. Estimates of the accuracy of the method are shown in Table I. Coefficients of variation averaged 6.7%. The reproducibility of the estimate of pirmenol in plasma was similar at all concentrations studied (Table II) and the coefficient of variation averaged 7.6%.

Application of the method for measuring blood and plasma pirmenol in a patient who received an infusion of the drug is illustrated in Fig. 3. Both concentrations reached a maximum at the end of the infusion and then declined biexponentially, with a terminal half-life of 9 h. Throughout, plasma concentrations were greater than those in blood such that the blood/plasma drug concentration ratio was 0.60 ± 0.06 S.D. in the samples analysed. In this patient, pirmenol concentration in a 24-h urine collection (total volume, 1645 ml) was 25.5 μg/ml. Thus of the 150 mg administered only 42 mg were recovered in the urine. This is consistent with metabolic transformation also being a route of elimination. In this particular patient ventricular ectopic beats disappeared during the infusion and began to return 4 h after drug administration when plasma pirmenol concentrations were 1.06 μg/ml. This

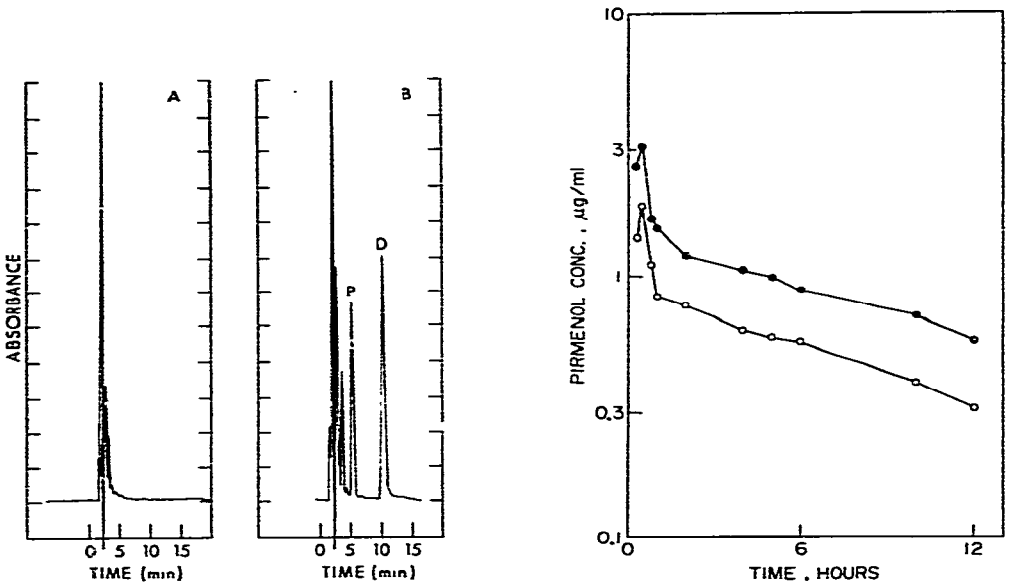

Fig. 2. Chromatograms of (A) control plasma and (B) plasma from a patient who had received pirmenol (P) to which the internal standard disopyramide (D) had been added. Each vertical division represents 0.01 a.u.f.s.

Fig. 3. Semi-logarithmic plot of plasma (•) and blood (○) concentrations of pirmenol during and after a 150-mg infusion of the drug over 30 min.

TABLE I

ESTIMATES OF THE ACCURACY OF THE METHOD

Biological fluid	Concentration range (µg/ml)	No. of studies	Coefficient of variation (%)	
			mean	range
Plasma	0.1—3.0	8	7.4	5.1—9.2
Blood	0.1—3.0	3	6.5	5.8—7.9
Urine	5.0—35.0	2	3.9	2 —5.8
All	0.1—35.0	13	6.7	2 —9.2

TABLE II

REPRODUCIBILITY OF THE METHOD FOR PLASMA

$n = 8$ in all cases.

Concentration (µg/ml)	NPHR*	Coefficient of variation (%)
0.10	0.91	5.8
0.25	0.93	7.5
0.50	0.99	6.5
1.00	1.01	9.6
1.50	1.03	9.5
2.00	1.08	6.4

*Mean peak height ratio.

is consistent with the effective plasma concentrations in arrhythmias following coronary artery ligation in the dog [1] and in blood superfused Purkinje fibers [2] determined using a fluorometric dye assay.

The presently HPLC method described for the analysis of pirmenol in biological samples is accurate within the putative range of effective concentrations and rapid enough that one person can manually analyse 15—20 samples during an average working day.

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