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Note

High-performance liquid chromatographic analysis of propafenone in human plasma samples

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Propafenone [2′-(2-hydroxy-3-propylamino-propoxy)-3-phenylpropiophenone] is a new antiarrhythmic drug which is undergoing clinical testing in several countries. In vitro studies have demonstrated that propafenone depresses $\dot{V}_{max}$ of the action potential, but does not alter the resting membrane potential [1]. Electrophysiological studies have shown that it prolongs sinus node recovery time, and lengthens the effective refractory period of the atrium and AV node [2]. Clinically, propafenone has been reported to be useful in suppressing chronic recurrent supraventricular and ventricular tachycardias, tachyarrhythmias and ectopic beats [3, 4].

In order to design optimal protocols to evaluate the clinical efficacy of propafenone, a better understanding of the pharmacokinetics and pharmacodynamics is needed. At present very little is known about the disposition kinetics of this drug. Prior to initiating pharmacokinetic and pharmacodynamic studies of propafenone, we have developed a simple and rapid high-performance liquid chromatographic procedure for measuring propafenone concentrations in biological fluids. This method is described and discussed in this report.

EXPERIMENTAL

Chemicals and reagents

Propafenone hydrochloride, the internal standard (Fig. 1) and ^{14}C-labeled propafenone were obtained from Knoll (Ludwigshafen, G.F.R.). Glass distilled acetonitrile was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.), AR grade heptane was purchased from Mallinckrodt (St. Louis, MO, U.S.A.), isoamyl alcohol from Eastman-Kodak (Rochester, NY, U.S.A.), and

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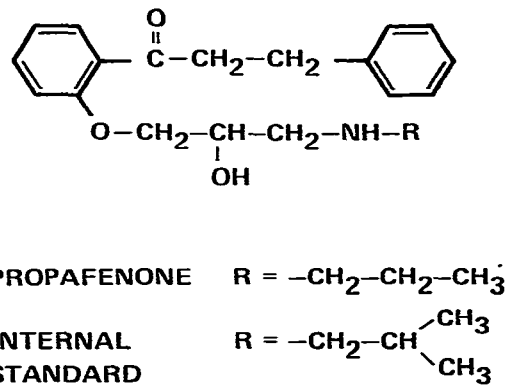

Fig. 1. Chemical structures of propafenone and the internal standard.

98% pure *n*-nonylamine from Aldrich (Milwaukee, WI, U.S.A.). All other chemicals and solvents were of reagent grade.

Instrumentation

A Waters Assoc. (Milford, MA, U.S.A.) M6000A Solvent Delivery System was used to pump the mobile phase through a Waters μBondapak CN column (30 cm × 3.9 mm); particle size was 10 μm. The flow-rate was adjusted to 1.6 ml/min which produced a precolumn pressure of 68 atm (1000 p.s.i.). The detector was a Schoeffel 770 UV—visible spectrophotometer with absorbance monitored at 209 nm.

Mobile phase

The mobile phase contained 25% acetonitrile and 75% 0.005 *M* potassium dihydrogen phosphate (pH 2.4). *n*-Nonylamine was added to the mixture to give a concentration of 0.02 *M*. The solution was filtered and degassed using vacuum.

Calibration standards

Calibration standards were prepared by adding weighed amounts of drug (reflecting amounts of free base) to distilled water. A few drops of 5 *N* hydrochloric acid (to a pH of 3.5) were added to ensure stability. These standards were prepared in appropriate dilutions to deliver between 10 and 250 ng of drug per 100 μl of solution. Calibration curves were prepared daily from these solutions. It was found that these solutions were stable, without observable decomposition, after being stored in the refrigerator for 9 months. Aliquots (100 μl) of each standard were pipetted into screw capped tubes. A 100-μl aliquot of internal standard (100 ng/100 μl) was added along with 0.5 ml of blank plasma. These samples were extracted along with the patient samples as described below.

Extraction procedure

Aliquots of 0.1—1 ml of patient plasma are added to screw-capped tubes along with 100 μl of the internal standard. To each tube are added 200 μl of 2 *N* sodium hydroxide (2/3 saturated with sodium chloride) and 2.0 ml of 1%

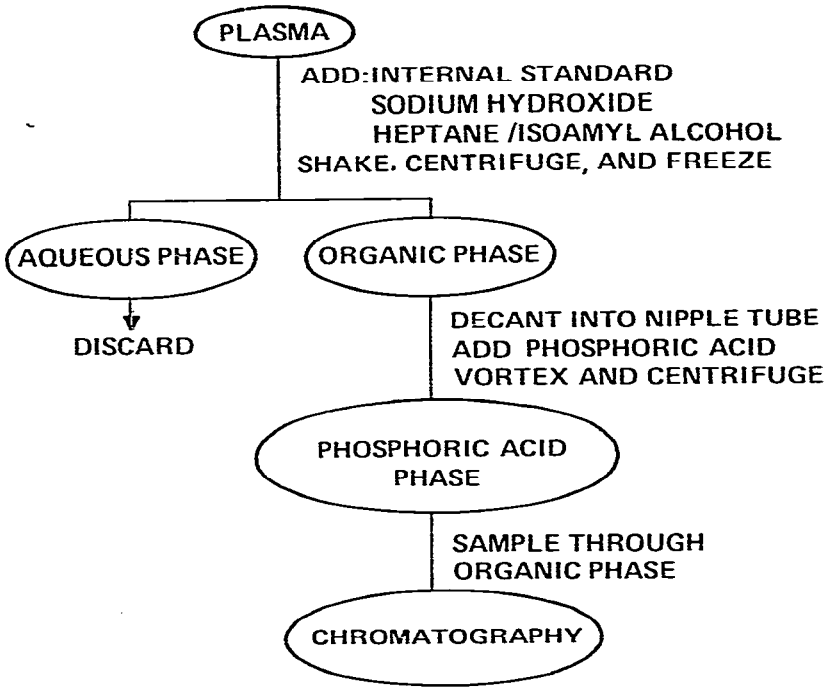

Fig. 2. Schematic outline of the sample preparation scheme used in the analysis of propafenone in plasma.

isoamyl alcohol in heptane. The tubes are capped and rocked on a Lab quake shaker for 10 min. After centrifugation to separate the organic and aqueous layers the tubes are placed briefly in a dry ice—acetone mixture to freeze the aqueous layer. The organic phase is then decanted into a clean nipple tube. To the nipple tube are added 200 μl of 0.2 *N* phosphoric acid. The tubes are then capped, shaken for 2 min on a Vortex mixer and centrifuged. A portion of the acid phase is then injected onto the column using a 50-μl syringe. This procedure is outlined schematically in Fig. 2.

RESULTS AND DISCUSSION

The retention times for propafenone and the internal standard are 5.9 and 7.0 min, respectively. The column temperature was not controlled, but generally stayed between 20°C and 28°C. Changes in temperature do influence the retention times, and this can be compensated for by changing the percentage of acetonitrile in the mobile phase.

Chromatograms of an extracted plasma sample from a healthy subject taking no medication and a sample from a patient who was being treated with propafenone are shown in Fig. 3. The patient was taking 150 mg of propafenone three times daily and the blood sample was drawn 1.5 h after a morning dose. The propafenone concentration at that time was 654 ng/ml. While no interferences have been observed in blank plasma samples from several sources, there was an unidentified peak eluting at 4 min in this patient's sample. This may be a metabolite of propafenone. This peak also has been observed in plas-

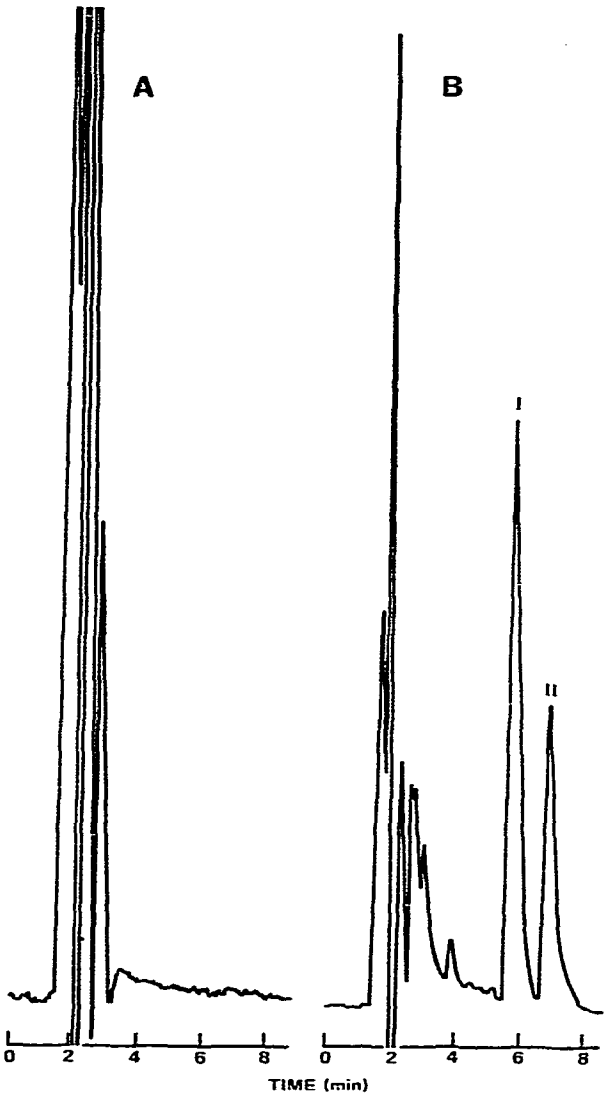

Fig. 3. Chromatograms of extracted patient plasma (A) not taking propafenone and (B) 1.5 h after taking 150 mg of propafenone orally. Peaks: I = propafenone; II = internal standard. The retention times of these two peaks are 5.9 and 7.0 min, respectively.

ma samples from other patients who were taking propafenone. The identity of the compound eluting at this time has not yet been determined. Another peak, eluting prior to propafenone, but with baseline separation, also has been noted in some patient samples. Whether this is also a metabolite of propafenone, or due to some other drug is not presently known.

Interference with this analysis by other drugs has been considered extensively, since patients taking antiarrhythmic drugs are often receiving numerous other medications. Most drugs studied do not interfere with this procedure, but a few interferences were observed. The following drugs do not interfere with

the analysis of propafenone: lidocaine, aldactone, warfarin, diltiazem, cimetidine, procainamide, atenolol, sotolol, quinidine, chlorothiazide, furosemide, digoxin and propranolol. The drugs which do interfere with the analysis of propafenone were nifedipine, verapamil and diazepam. However, it is not likely that the slow channel blockers, verapamil and nifedipine, would be administered concurrently with propafenone. The retention time of diazepam was 5.4 min, which was very close to that of propafenone. These two peaks can be further separated by slowing down the chromatography, but this greatly limits the utility of this method for handling large numbers of samples.

Calibration curves are linear up to 500 ng. The usual range employed has been 25—500 ng, since this includes the range of most of the samples analyzed to date. The use of less internal standard permits the analysis of concentrations as low as 5 ng/ml. The daily fluctuation in slope of the standard curve is slight; the coefficient of variation of the slope of five different standard curves was only 3.5%. The y-axis intercepts are not different from zero.

The efficiency of the extraction procedure was determined using [^{14}C] propafenone as tracer. It was found that 78% of the drug was extracted into the final acid phase, 18% was left in the plasma, 1% was in the organic phase and 3% was unaccounted for. The percent extracted was constant with a standard deviation of only 1.6% ($n = 4$).

Using labeled propafenone, the partitioning between red blood cells and plasma water was also examined. A plasma/blood concentration ratio of 1.12 was observed. This suggests that propafenone is bound to plasma proteins, and only the free drug partitions into the red cells.

To increase the range of analysis, it is often desirable to use varied volumes of patient plasma. Larger volumes may be employed for low-concentration samples and small volumes for high-concentration samples. Varied volumes can be employed if plasma volume does not affect the extraction of the drug and alter the relationship between the drug and internal standard. In order to test this, spiked plasma samples were analyzed. The extracted volumes of plasma ranged from 0.1 to 1.0 ml. The coefficient of variation was 1.5% indicating that sample size does not affect the relative extraction efficiences of propafenone and the internal standard.

The reproducibility of this procedure was evaluated by analyzing several samples at specific plasma concentrations (Table I). A range of 5—150 ng/ml was studied. The greatest standard deviation was observed with the 50 ng/ml samples, but this only amounted to 3.6 ng/ml. Despite the large coefficient of

TABLE I

REPRODUCIBILITY OF A GIVEN PLASMA CONCENTRATION

Concentration (ng/ml)	n	C.V. (%)
5	6	10.6
10	6	8.7
50	12	7.1
100	6	0.7
150	6	2.1

variation observed with the 5 ng/ml samples, this is only a standard deviation of 0.53 ng/ml. We have observed that this method is very stable and quite reproducible from day to day.

The method reported here has sufficient sensitivity for both therapeutic monitoring and pharmacokinetic studies. While it is specific for propafenone, three drug interferences have been identified. The method is relatively simple and rapid, allowing analysis of up to 40 samples daily.

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