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Note

High-performance liquid chromatographic analysis of lorcainide and its active metabolite, norlorcainide, in human plasma

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Lorcainide is a new antiarrhythmic drug which is effective for the treatment of premature ventricular contractions [1] and ventricular tachycardia [2]. It is effective following both oral and intravenous administration. Lorcainide undergoes a saturable presystemic elimination following oral administration, and an active metabolite, norlorcainide, accumulates in the plasma to concentrations greater than those of the parent drug [3]. Norlorcainide has been shown to possess antiarrhythmic properties similar to those of lorcainide [4]. Due to the variability in the first-pass extraction and the accumulation of the active metabolite, it is desirable to measure lorcainide and norlorcainide plasma concentrations during clinical trials and as a therapeutic aid.

A gas chromatographic method for the analysis of lorcainide and norlorcainide has been published previously [5]. The method employed electron-capture detection and was sensitive to a lower limit of 10 ng/ml of lorcainide or norlorcainide in plasma. The method described here is a high-performance liquid chromatographic procedure using a reversed-phase system with UV absorbance detection. Our method is sensitive to a lower limit of detection of 5 ng/ml of lorcainide or norlorcainide in plasma. The internal standard employed is the calcium antagonist D-600 which is structurally dissimilar from lorcainide (see Fig. 1), but has similar solubility and chromatographic and absorptive characteristics.

EXPERIMENTAL

Chemicals and reagents

Lorcainide HCl {N-(4-chlorophenyl)-N-[1-(1-isopropyl)-4-piperidinyl] benzeneacetamide hydrochloride} and norlorcainide [N-(4-chlorophenyl)-N-(4-

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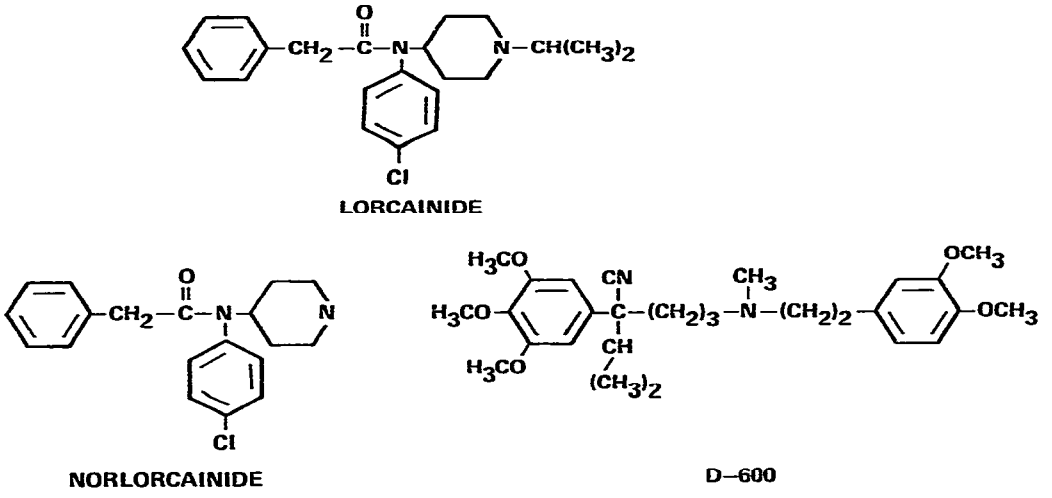

Fig. 1. Structures of lorcainide, norlorcainide, and D-600.

piperidinyl) benzeneacetamide] were provided by Janssen Pharmaceutical (New Brunswick, NJ, U.S.A.). The internal standard D-600 {α-isopropyl-α-[(N-methyl-N-homoveratryl)-α-aminopropyl]-3,4,5-trimethoxyphenylacetonitrile hydrochloride} was obtained from Knoll (Ludwigshafen, G.F.R.). Glass distilled acetonitrile was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). All other solvents were of reagent grade.

Instrumentation and chromatographic conditions

A Varian Model 8500 high-pressure liquid chromatographic pump was employed to pump solvent through a Waters Assoc. (Milford, MA, U.S.A.) μ-Bondapak phenyl reversed-phase column (30 cm × 3.9 mm I.D.; particle size 10 μm). The flow-rate was 80 ml/h which produced a precolumn pressure of 68 atm (1000 p.s.i.). The detector was a Schoeffel Model SF 770 variable-wavelength absorbance detector. The detector was set for absorbance at a wavelength of 196 nm. A dual-pen Houston-Omniscribe recorder was used at 10 and 20 mV outputs. Chart speed was 0.5 cm/min.

Mobile phase preparation

The mobile phase consisted of acetonitrile—phosphate buffer (2:3). The phosphate buffer was an 0.02 *M* solution of KH_2PO_4 with the pH adjusted to 2.3 by the dropwise addition of approximately 1.9 ml of H_3PO_4 per l of buffer. The mixture was filtered and degassed prior to use.

Extraction procedure

The extraction procedure is outlined in Fig. 2. PTFE-lined, screw-capped tubes are initially rinsed and wetted with heptane—isoamyl alcohol (95:5). Plasma (0.2—2.0 ml) and 100 μl of aqueous internal standard solution (200 ng) are added to tubes. The plasma is made basic with the addition of 200 μl of 2 *N* NaOH, which is 67% saturated with NaCl, and 5 ml of a mixture of heptane—isoamyl alcohol (95:5) is added. The samples are mixed by gentle rocking on a

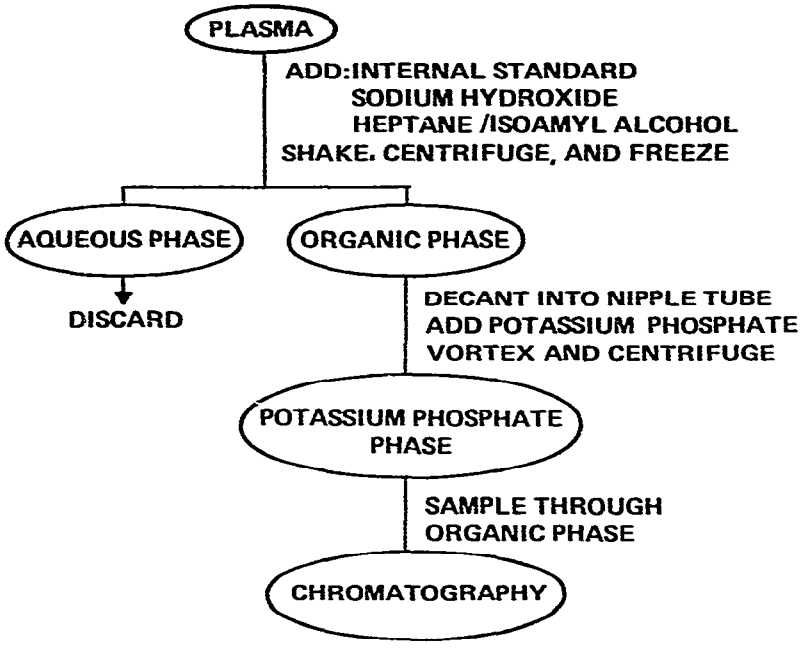

Fig. 2. Schematic outline of the sample preparation used in the analysis of lorcainide and norlorcainide in plasma.

mechanical shaker for 10 min and then centrifuged for 15 min. The aqueous phase is frozen by placing the tubes in a mixture of acetone—dry ice for a minute, and the organic phase is decanted into a clean tube with an elongated cone at its base of approximately 50 μl capacity. To this tube are added 20 μl of 0.02 *M* KH_2PO_4, which has been adjusted to a pH of 1.5 with H_3PO_4. The sample is shaken on a vortex mixer for 90 sec and centrifuged to separate the aqueous and organic layers. An aliquot (10 μl) of the aqueous phase is injected into the chromatograph using a 25-μl syringe.

Preparation of calibration standards

Aqueous stock solutions containing lorcainide and norlorcainide at concentrations ranging from 5 to 514 ng per 100 μl are used for preparing standard curves. These are stored at 4° C with no detectable decomposition of lorcainide or norlorcainide over a three-month period.

RESULTS AND DISCUSSION

The retention times for norlorcainide, lorcainide and the internal standard are 4.3, 6.0 and 7.7 min, respectively. Chromatograms of an extract of plasma from a subject not taking lorcainide (A) and an extract of a plasma sample from a patient who was taking lorcainide (B) are shown in Fig. 3. A structural analog of lorcainide, N-(4-chlorophenyl)-N-[1-(3-methylbutyl)-4-piperidinyl] benzeneacetamide, was also evaluated for use as an internal standard; it eluted with a retention time of over 10 min. There also appeared to be more variation in the analysis of repeated samples when this compound was employed. Due to

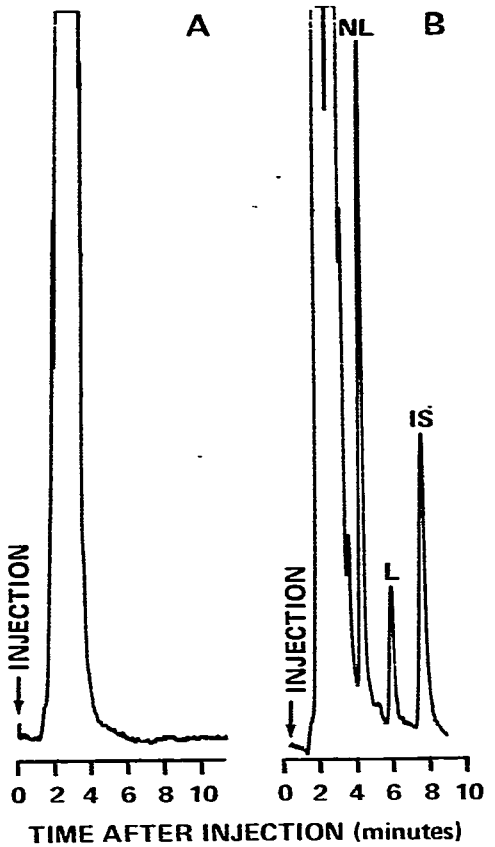

Fig. 3. Chromatograms of extracted patient plasma (A) not taking lorcainide and (B) 8 h after taking 100 mg of lorcainide orally. Peaks: L, lorcainide, measured 32 ng/ml; NL, norlorcainide, 123 ng/ml and IS, internal standard, D-600.

the slow elution and the problem with variability, D-600 was employed as a more desirable internal standard. The chromatogram shown in Fig. 3B was from a blood sample drawn 8 h following administration of a 100-mg oral dose of lorcainide. The measured concentrations of lorcainide and norlorcainide were 32 ng/ml and 123 ng/ml, respectively.

The chromatography was sensitive to slight changes in the mobile phase composition. Increasing the molarity of the phosphate buffer decreased the retention times and subsequently the separation of the three components. The acidity of the phosphate buffer also greatly influenced the retention times, with the retention times decreasing with a decrease in pH. Since a pH of 2 is the lowest recommended pH for a bonded-phase column, a pH of 2.3 was employed. This pH maximized the chromatography, producing sharp symmetrical peaks with good baseline separation. Under these conditions, samples could be injected every 9 min.

Rinsing the tubes and caps used for extraction with the organic solvent was found to improve the precision of the assay. When they were not rinsed with the heptane–isoamyl alcohol solvent, poor reproducibility was observed with coefficients of variability of greater than 10%. The extraction efficiencies of

lorcainide and norlorcainide were determined by comparing peak heights of extracted and directly injected ssmples. The recovery of lorcainide was 38 ± 0.6%, and for norlorcainide it was 41 ± 1.0%.

Since patients receiving antiarrhythmic drug therapy often are given other medications concurrently, interference from other compounds with the analysis of lorcainide was evaluated. Several drugs were added to plasma samples in quantities representative of therapeutic or higher concentrations. The following drugs were evaluated: lidocaine, quinidine, propranolol, metroprolol, digoxin, verapamil, chlorothiazide, and furosemide. A peak at 3.8 min was observed with propranolol, but this did not cause any major interference. Verapamil, however, eluted as a peak at 7.0 min, which was not totally separated from the internal standard. In the event that lorcainide plasma concentrations need to be measured in the presence of verapamil, either the chromatography can be slowed down to produce a better separation, or the methyl-butyl analog of lorcainide can be utilized as an internal standard.

Standard curves were prepared by adding known amounts of drug and metabolite to blank blood or plasma samples and determining the peak height ratios (lorcainide or norlorcainide/internal standard). The standard curves were linear over a range of from 5 to 500 ng/ml and extrapolated through the

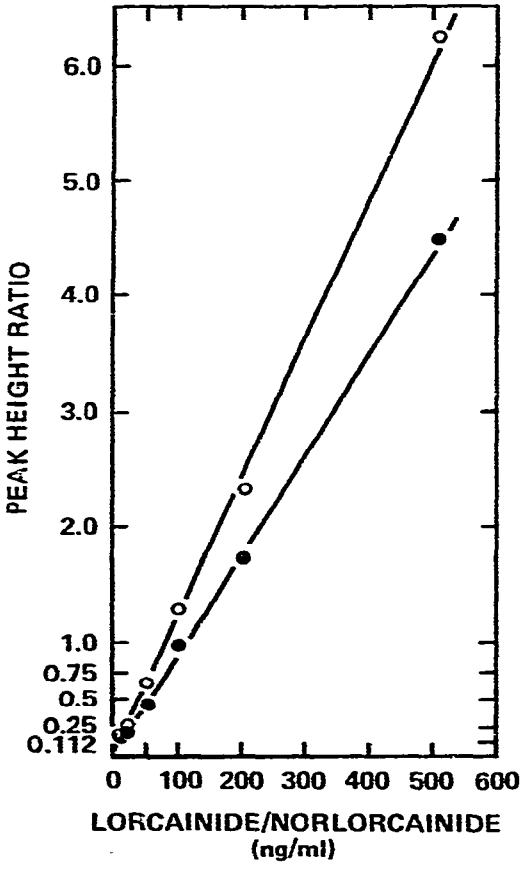

Fig. 4. Standard curve for lorcainide and norlorcainide extracted simultaneously was linear over a range from 5 to 500 ng/ml of plasma and extrapolated through the origin.

origin. A typical standard curve for lorcainide and norlorcainide analyzed simultaneously from plasma is shown in Fig. 4. The average coefficient of variation from eight standard curves was 6.03 ± 1.14% for lorcainide and 5.16 ± 2.34% for norlorcainide.

The lowest concentration standard which was routinely employed for the calibration curve was 11 ng/ml for both lorcainide and norlorcainide. Only on rare occasions were samples containing less than 10 ng/ml encountered. The lower limit of sensitivity of this method is 5 ng/ml.

Reproducibility was evaluated by extracting and analyzing replicate plasma samples containing 11, 51, and 514 ng/ml of lorcainide and norlorcainide. These data are summarized in Table I. The coefficients of variation (C.V.) for lorcainide at 11, 51, and 514 mg/ml were 5.21, 1.09, and 2.37%, respectively. The C.V. values for norlorcainide at each concentration were 4.51, 8.80, and 3.59%, respectively.

TABLE I

REPRODUCIBILITY AT GIVEN PLASMA CONCENTRATIONS $N = 5$

Concentration (ng/ml)		C.V. (%)
Lorcainide	Norlorcainide	
11	—	5.21
51	—	1.09
514	—	2.37
—	11	4.51
—	51	8.80
—	514	3.59

Since it may be desirable to extract plasma samples on a day prior to chromatographic analysis, the stability of lorcainide and norlorcainide was investigated when left in the acid phase after completing the extraction. Samples left in acid at 4°C or room temperature for up to seven days showed no signs of decomposition, suggesting that lorcainide, norlorcainide and the internal standard are stable under these conditions for at least a week.

The method described here for the quantitative determination of lorcainide and norlorcainide in plasma is simple and rapid and allows the daily analysis of 25—30 plasma samples.

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