Journal of Chromatography, 231 (1982) 478—484
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1332

Note

Rapid and simple method for determination of lorcainide, a new antiarrhythmic drug, and its major metabolite, norlorcainide, by high-performance liquid chromatography

VERNE SIMON and PITAMBAR SOMANI*

Division of Clinical Pharmacology, Departments of Pharmacology and Medicine, Medical College of Ohio, C.S. 10008, Toledo, OH 43699 (U.S.A.)

(First received January 29th, 1982; revised manuscript received April 1st, 1982)

Lorcainide is a new antiarrhythmic drug [1, 2] which is being clinically evaluated for the management of ventricular dysrhythmias in this country [3]. Since antiarrhythmic drugs in general have a relatively narrow therapeutic index, it is quite important to measure their plasma concentrations and this would also be the case for lorcainide in patients receiving this new drug. It is also important to determine whether or not one or more metabolites are present in the blood of such patients. Lorcainide and its major metabolite, norlorcainide, have been identified and quantitated by gas chromatography [4, 5]. Yee and Kates [6] recently described a high-performance liquid chromatographic (HPLC) method to determine these drugs; however, the extraction efficiency from the plasma in their method was only 38% for lorcainide and 41% for norlorcainide. Their procedure also involved detection at 196 nm, which is not possible with any HPLC system equipped with the more common fixed UV detector at 254 nm. We report an improvement in the HPLC method for rapid determination of plasma lorcainide and norlorcainide which would make therapeutic drug level monitoring more practical.

EXPERIMENTAL

Instrument and chromatographic conditions

All HPLC components were manufactured by Waters Associates and included: 6000A solvent delivery system, U6K injector, and Model 440 absorbance detector with wavelength fixed at 254 nm. The wave forms were recorded on a Houston OmniScribe Model B 5217-1 strip chart recorder. The column was Waters μBondapak phenyl reversed-phase, 30 cm × 3.9 mm

0378-4347/82/0000—0000/$02.75 © 1982 Elsevier Scientific Publishing Company

I.D., with particle size of 10 μm. The flow-rate was 1 ml/min which produced a precolumn pressure of 68.95 bars. Detection was at 254 nm with sensitivity at the highest setting, 0.005. The chart speed was 0.5 cm/min.

Chemicals and reagents

Lorcainide hydrochloride {N-(4-chlorophenyl)-N-[1-(1-isopropyl)-4-piperidinyl]benzeneacetamide hydrochloride} and norlorcainide [N-(4-chlorophenyl)-N-(4-piperidinyl)benzeneacetamide], as the free base, were provided by Janssen Pharmaceutical (New Brunswick, NJ, U.S.A.). D-600 or gallopamil hydrochloride, {α-isopropyl-α-[(N-methyl-N-homoveratryl)-α-aminopropyl]-3,4,5-trimethoxyphenylacetonitrile hydrochloride}, the internal standard, was obtained from Knoll (Ludwigshafen, G.F.R.). Acetonitrile and *n*-pentane (HPLC grade) were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). Isopropanol (spectrophotometric grade) was obtained from Mallinckrodt (St. Louis, MO, U.S.A.); all other chemicals were reagent grade.

Mobile phase preparation

For 2 l of mobile phase, 5.44 g of potassium dihydrogen phosphate were dissolved in ca. 1000 ml of distilled water in a 2-l beaker; 750 ml of acetonitrile were added and the pH was adjusted to 2.3 using 42% phosphoric acid. The mobile phase was then transferred to a 2-l volumetric flask and distilled water was added to the 2-l mark. A type FH 0.5-μm Millipore filter was wetted by filtration of a few milliliters of acetonitrile and the mobile phase was then filtered, discarding the first small portion.

Extraction procedure

Method I. Glass tubes with PTFE-lined screw caps were rinsed with *n*-pentane. A small amount (1—3 ml) of either control or patient's plasma was added along with D-600 (1.6 μg). The tubes were gently vortexed (1 sec). A solution containing 2 *M* sodium hydroxide and 4 *M* sodium chloride was added (200 μl) along with *n*-pentane (5 ml) and isopropanol (200 μl). The tubes were placed on a nearly horizontal wheel and rotated at 40 rpm for 20 min followed by bench-top centrifugation for 10 min. The plasma layer was frozen by immersion in dry ice—ethanol and the organic layer decanted into conical centrifuge tubes and evaporated to dryness in warm water (initial temperature 45°C, final temperature 80°C). To the cooled tubes, 0.8 *M* phosphoric acid (150 μl) and *n*-pentane (750 μl) were added. After vigorous vortexing (90 sec) the aqueous layer was frozen in dry ice—ethanol, and the organic layer was removed by aspiration. The pH of the aqueous layer was adjusted to 6.6 by addition of 3 *M* sodium hydroxide (50 μl). The solution was filtered through a 0.2 μm polytetrafluoroethylene microfilter (Anspec, Ann Arbor, MI, U.S.A.), and a 100—180 μl portion of the solution was injected into the HPLC system.

Method II. Extraction method II was quite similar to method I except that the *n*-pentane—isopropanol layer (5 ml) was extracted directly with 0.8 *M* phosphoric acid (150 μl) without evaporation. The aqueous layer was treated as in method I except that filtration was not necessary.

Preparation of calibration standards

Aqueous solutions containing equal amounts of 0.025 or 0.005 μg/μl lorcainide hydrochloride and norlorcainide (free base) were used in establishing standard curves. The solutions contained $2.4 \cdot 10^{-4}$ and $4.9 \cdot 10^{-5}$ M phosphoric acid, respectively, as an aid in solubilization of the norlorcainide free base. The D-600 solution used contained 0.04 μg/μl aqueous D-600 hydrochloride. Throughout this paper the concentration of norlorcainide refers to the free base while that of lorcainide and D-600 refers to the hydrochloride salt.

RESULTS AND DISCUSSION

The retention times for norlorcainide, lorcainide and D-600 on the μBondapak phenyl column were 7.0, 9.8 and 13.2 min, respectively. The retention times were strongly dependent on solvent composition and pH (see also Yee and Kates [6]). The chromatogram of plasma of a patient being treated with oral lorcainide is shown in Fig. 1 and it may be seen that the three drugs

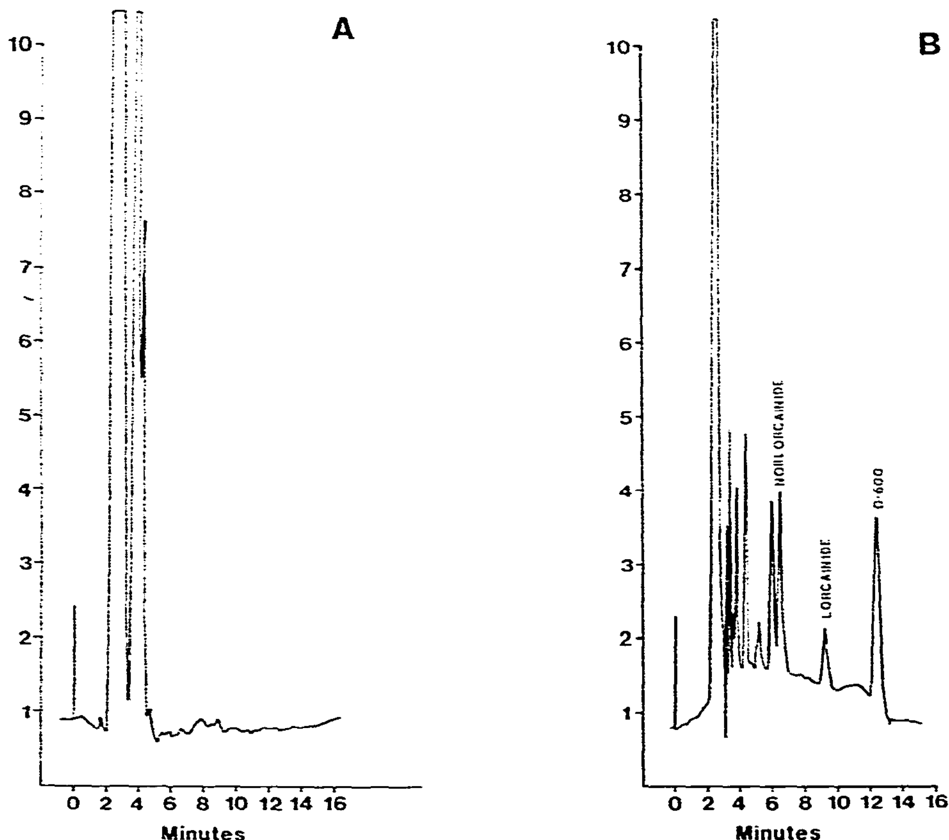

Fig. 1. Chromatogram showing the blank plasma (A) and the peaks for norlorcainide, lorcainide and the internal standard, D-600, in the plasma of a 72-year-old white male patient being treated with 300 mg/day oral lorcainide (B). The extract was injected into the chromatograph at time 0 and the retention time in minutes is indicated along the horizontal axis.

can be easily identified in this patient. The standard curves of lorcainide and norlorcainide were established by adding the two drugs to the plasma from healthy volunteers in concentrations from 0.06 μg/ml to 0.9 μg/ml, followed by the extraction procedure as described above by both methods I and II. Peak height ratios of lorcainide or norlorcainide to D-600 (h_L/h_D or h_N/h_D) were determined. Linear regression, with predetermined zero y-intercept, of the drug concentration (y-axis) on peak height ratio (x-axis) yielded the following equations for method I

$$[N] = (1.117 \pm 0.018 \text{ S.D.})\, h_N/h_D \ (n = 37) \quad (1)$$

$$[L] = (1.307 \pm 0.012 \text{ S.D.})\, h_L/h_D \ (n = 37) \quad (2)$$

and for method II

$$[N] = (1.262 \pm 0.016 \text{ S.D.})\, h_N/h_D \ (n = 33) \quad (3)$$

$$[L] = (1.319 \pm 0.012 \text{ S.D.})\, h_L/h_D \ (n = 33) \quad (4)$$

where [N] = calculated plasma concentration of norlorcainide free base (μg/ml), and [L] = calculated plasma concentration of lorcainide hydrochloride (μg/ml). All four of the above equations are based on the peak height (h_D) of 1.6 μg D-600 per ml plasma added prior to the extraction procedure. The coefficients of the peak height ratios were proportional to the quantity of internal standard added.

In order to evaluate the assay results of the HPLC, eqns. 1—4 were used to generate calculated versus actual values for lorcainide and norlorcainide concentrations for both extraction methods. Linear regression analysis of calculated concentration on actual concentration was carried out in all four cases, and the results for method I are shown in Figs. 2 and 3. Excellent correlation between actual and measured concentration was found for both lorcainide and norlorcainide and, based upon these results, the four equations for calculated versus actual concentration are as follows:
Extraction method I

$$[N]_{\text{calcd.}} = 0.013 + 0.97\,[N]_{\text{actual}};\ r^2 = 0.954$$

$$[L]_{\text{calcd.}} = 0.003 + 0.99\,[L]_{\text{actual}};\ r^2 = 0.992$$

Extraction method II,

$$[N]_{\text{calcd.}} = 0.0022 + 0.96\,[N]_{\text{actual}};\ r^2 = 0.987$$

$$[L]_{\text{calcd.}} = 0.014 + 1.02\,[L]_{\text{actual}};\ r^2 = 0.994$$

Reproducibility of the procedure was evaluated by replicate analysis of five plasma samples containing equal concentrations of lorcainide and norlorcainide at each of three different concentrations. For extraction method I,

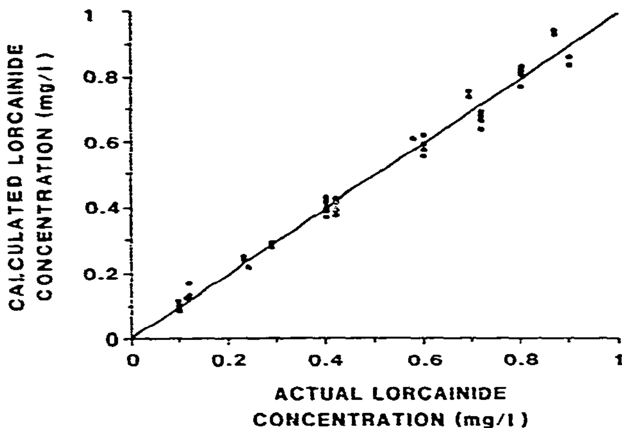

Fig. 2. Linear regression analysis of the actual versus calculated lorcainide concentration. The amount of lorcainide added to control plasma is shown along the horizontal axis and the calculated concentration from the peak height ratio (eqn. 2) is shown along the vertical axis. Note that an excellent correlation ($r^2 = 0.992$) exists for actual versus calculated concentration of the drug ($y = 0.003 + 0.99x$; $P < 0.001$).

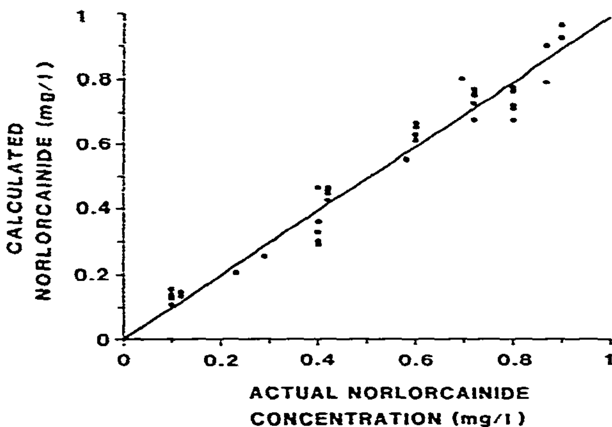

Fig. 3. Linear regression analysis of the actual versus calculated norlorcainide concentration. The amount of norlorcainide added to the control plasma is shown along the horizontal axis and the calculated concentration from the peak height ratio (eqn. 1) is shown along the vertical axis. Note that an excellent correlation ($r^2 = 0.954$) exists for actual versus calculated concentration of the drug ($y = 0.013 + 0.97x$; $P < 0.001$).

studies were done at concentrations of 0.1, 0.4 and 0.8 μg/ml and the coefficients of variation for the calculated concentrations were 23.2, 20.8 and 5.8%, respectively, for norlorcainide and 12.1, 4.9 and 2.5%, respectively, for lorcainide. Similar replicate studies were done at concentrations of 0.06, 0.3 and 0.9 μg/ml for extraction method II of the two drugs and coefficients of variation of 9.5, 5.0 and 5.2%, respectively, for norlorcainide and 3.4, 1.6 and 4.9%, respectively, for lorcainide were found. The results of the reproducibility studies are summarized in Table I. The poorer reproducibility observed for method I may be due to the injection of only some of the samples without prior filtration.

TABLE I

REPRODUCIBILITY OF LORCAINIDE/NORLORCAINIDE DETERMINATIONS IN HUMAN PLASMA

Method I, n = 37; method II, n = 33.

Concentration of lorcainide or norlorcainide (μg/ml)	C.V.* (%)			
	Norlorcainide		Lorcainide	
	Method I	Method II	Method I	Method II
0.06	—	9.5	—	3.4
0.1	23.2	—	12.1	—
0.3	—	5.0	—	1.6
0.4	20.8	—	4.9	—
0.8	5.8	—	2.5	—
0.9	—	5.2	—	4.9

*C.V. = coefficient of variation, as determined by the equation $C.V. = \frac{S.D.}{mean} \times 100$.

The extraction efficiency was determined by comparing the peak height after injection of the entire aqueous extract of the drug with the peak height for direct injection of the same quantity of the drug in a standard aqueous solution (Fig. 4). The recovery of lorcainide and norlorcainide was 75 ± 11% (n = 37) and 49 ± 11% (n = 37), respectively, for extraction method I, and

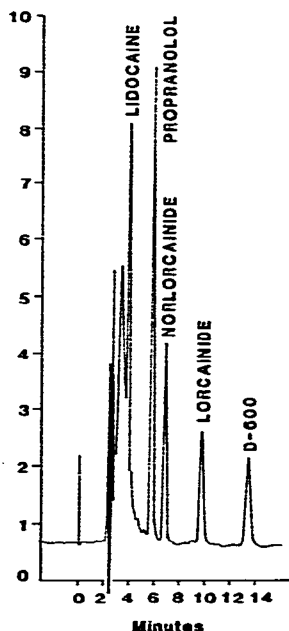

Fig. 4. Chromatogram of an aqueous solution containing a mixture of lidocaine, propranolol, norlorcainide, lorcainide and D-600. It may be noted that the peaks for each of the five drugs can be easily identified.

65 ± 5% (n = 33) and 43 ± 6% (n = 33), respectively, for extraction method II. Yee and Kates [6] reported extraction efficiency of 38 ± 0.6% and 41 ± 1% for lorcainide and norlorcainide, respectively, by their procedure using heptane—isoamyl alcohol.

Since with detection at 254 nm one must work at the highest sensitivity setting (0.005), a 2.0-fold (extraction method I) or 1.7-fold (extraction method II) increase in lorcainide recovery over the previously available method is an important advantage.

Extraction by method I permits daily assay of about 18 samples, while extraction by method II permits daily assay of about 24 samples. Because method II is faster without too great a loss in sensitivity, we now routinely use extraction method II for determination of lorcainide and norlorcainide in our patient population. If sample volume permits, the use of 2 or 3 ml of plasma is helpful. Concentrations as low as 0.008 µg/ml can be measured by our method, although the usual therapeutic concentrations of lorcainide and norlorcainide in patients receiving 200—400 mg per day of the parent drug range between 0.1 and 1 µg/ml for lorcainide and 0.1 and 1.5 µg/ml for norlorcainide, respectively. These results demonstrate that our procedure can be used easily in most clinical laboratories equipped with a standard HPLC system with detection at 254 nm for therapeutic drug monitoring of this new antiarrhythmic agent.

ACKNOWLEDGEMENT

We wish to thank Ms. Debra LeBarr for excellent secretarial help in preparation of this manuscript.

REFERENCES

1 H. Kesteloot and R. Stroobandt, Arch. Int. Pharmacodyn. Ther., 230 (1977) 255.
2 W.K. Amery, J.J.P. Heykants, R. Xhonneux, G. Towse, P. Oettel, D.A. Gough and P.A.J. Janssen, Acta Cardiol., 36 (1981) 207.
3 P. Somani, Amer. J. Cardiol., 48 (1981) 157.
4 U. Klotz, P. Müller-Seylitz and P. Heimburg, Clin. Pharmacokin., 3 (1979) 407.
5 R. Woestenborghs, M. Michiels and J. Heykants, J. Chromatogr., 164 (1979) 169.
6 Y.-G. Yee and R.E. Kates, J. Chromatogr., 223 (1981) 454.