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SIMULTANEOUS GAS CHROMATOGRAPHIC DETERMINATION OF LORCAINIDE HYDROCHLORIDE AND THREE OF ITS PRINCIPAL METABOLITES IN BIOLOGICAL SAMPLES

ROBERT WOESTENBORGHS*, MARCEL MICHIELS and JOS HEYKANTS

Department of Drug Metabolism, Janssen Pharmaceutica, 2340 Beerse (Belgium)

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SUMMARY

A method is described for the determination of the antiarrhythmic drug lorcainide hydrochloride and its three main metabolites in plasma, urine, faeces and tissues from man and animals. The procedure involves the extraction of the parent drug, its metabolites and the internal standard from the biological materials at different alkaline pH values, back-extraction into sulphuric acid and re-extraction into the organic phase (heptane—isoamyl alcohol). After silylation of the different phenolic and the N-dealkylated metabolites, analyses were carried out by automated gas—liquid chromatography with electron-capture detection. The method has a sensitivity limit of 5 ng for lorcainide, and 10—20 ng for the various metabolites, per millilitre of plasma.

The method was applied to urine, faeces, plasma and tissue samples from man and animals. It was also suitable for automatic sample analysis.

INTRODUCTION

Lorcainide hydrochloride, N-(4-chlorophenyl)-N-[1-(1-methylethyl)-4-piperidinyl]benzeneacetamide monohydrochloride (I; Fig. 1), is a promising new antiarrhythmic agent, effective against ventricular arrhythmias and atrial fibrillation [1—4]. Studies concerned with its metabolic fate have identified the N-dealkylated and three phenolic metabolites in human plasma [5, 6].

This paper describes a sensitive procedure for the simultaneous determination of both lorcainide and the main metabolites in plasma, urine, faeces and tissue samples. The method was used to obtain more detailed information about the pharmacokinetics of the drug in man and animals [7, 8].

*To whom correspondence should be addressed.

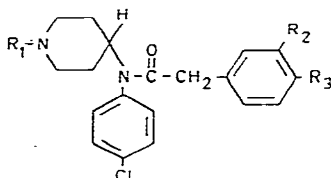
EXPERIMENTAL

Standards and reagents

Lorcainide hydrochloride, nor-lorcainide or N-(4-chlorophenyl)-N-(4-piperidinyl)benzeneacetamide (II), N-(4-chlorophenyl)-4-hydroxy-N-[1-(1-methylethyl)-4-piperidinyl] benzeneacetamide (III), N-(4-chlorophenyl)-4-hydroxy-3-methoxy-N-[1-(1-methylethyl)-4-piperidinyl[benzeneacetamide (IV), and the internal standard, N-(4-chlorophenyl)-N-[1-(3-methylbutyl)-4-piperidinyl] benzeneacetamide hydrochloride (V), were all originally synthesized in our research laboratories and were of analytical grade. Chemical structures are shown in Fig. 1.

Spectrophotometric grade *n*-heptane and acetonitrile were used; methanol and isoamyl alcohol were of analytical grade. The silylating reagent consisted of N,O-bis(trimethylsilyl)acetamide (BSA), to which 1% of trimethylchlorosilane (TMCS) had been added as a catalyst (Aldrich-Europe, Beerse, Belgium).

The inorganic reagents were prepared in double-distilled water. A borate buffer (pH 8.5) was prepared by adding 15.2 ml of 0.1 *M* hydrochloric acid to 50 ml of 0.025 *M* sodium borate decahydrate (borax). A final volume of 100 ml was prepared.



Compound	R_1	R_2	R_3
R 15889 (I)	$CH-CH_3$ CH_3	H	H
R 15665 (II)	H	H	H
R 39611 (III)	$CH-CH_3$ CH_3	H	OH
R 41853 (IV)	$CH-CH_3$ CH_3	OCH_3	OH
R 17251 (V)	$CH_2-CH_2-CH-CH_3$ CH_3	H	H

Fig. 1. Chemical structures of lorcainide (R 15889), its principal metabolites (R 15665, R 39611 and R 41853) and the internal standard (R 17251).

Standard solutions

A combined standard solution containing 25 mg of all synthesized drugs (I, II, III and IV) as the free bases was prepared in 25 ml of methanol.

A 10-ml volume of the internal standard solution was also prepared at a

concentration of 1 mg/ml. To spike the samples with the internal standard, an aliquot of this stock solution was further diluted to 2 μg/ml.

All the solutions were stored in a refrigerator at 4° prior to use.

Extraction procedure

Plasma. Plasma samples (1 ml) were pipetted into 15-ml glass centrifuge tubes and spiked with 0.2 μg of the internal standard. The solution was buffered with 2.5 ml of the borate buffer solution (pH 8.5) and 4 ml of heptane—isoamyl alcohol (95:5, v/v) were added. The tubes were carefully rotated for 10 min (35 rpm, Cenco rotary mixer) and then centrifuged (5 min, 1000 *g*). The upper organic layer was transferred to a second centrifuge tube, containing 2 ml of 0.05 *M* sulphuric acid. The organic layer was removed and discarded after shaking and centrifugation. After repetition of this step, the plasma in the first tube was made more alkaline by adding a few drops of 1 *M* sodium hydroxide (pH 11) and extracted twice with 4 ml of the organic solvent mixture. Both upper organic layers were transferred to the second tube and discarded after extraction with the sulphuric acid. The remaining acidic phase was made basic with concentrated ammonia (pH 10) and extracted twice with 2 ml of the heptane—isoamyl alcohol mixture. The combined organic layers were evaporated to dryness under a stream of nitrogen in a water bath at 60°.

Other samples. Human urine and faeces as well as different animal tissues were similarly processed.

Urine samples of 0.1 ml were processed by the present extraction procedure.

Faeces were homogenized (1:5, w/v) in methanol, using an Ultra-turrax TP 18/2 homogenizer. After centrifugation, the precipitates were washed twice with methanol and centrifuged again. The various supernatants of each faeces sample were mixed and the volume was measured. One millilitre of each of these faeces extracts was then submitted to the extraction procedure.

Tissue samples of 2 g were homogenized in 100-ml centrifuge tubes, containing 7.5 ml of distilled water and 2.5 ml of the borate buffer solution. The homogenates were processed as described above.

The extraction procedure is outlined in Fig. 2.

Calibration procedure

Samples of control plasma (1 ml) were spiked with lorcainide and its metabolites at concentrations ranging from 0.01 to 3 μg/ml, and with the internal standard at a fixed concentration of 0.2 μg/ml. The samples were taken through the extraction procedure described previously.

Apparatus

All the analyses were performed on a Varian Model 3700 gas chromatograph equipped with a Varian Model 8000 automatic sample injector and a pulse-modulated constant-current ^{63}Ni electron-capture detector.

The glass column (200 × 0.2 cm) was packed with 3% OV-22 on 80—100 mesh Supelcoport (Supelco, Bellefonte, Pa., U.S.A.). The column temperature was 260° and the injector and detector temperatures were 290° and 320°, respectively. Nitrogen was used as a carrier gas at a flow-rate of 40 ml/min.

Area integrations, calculations and plotting of the chromatograms as well as

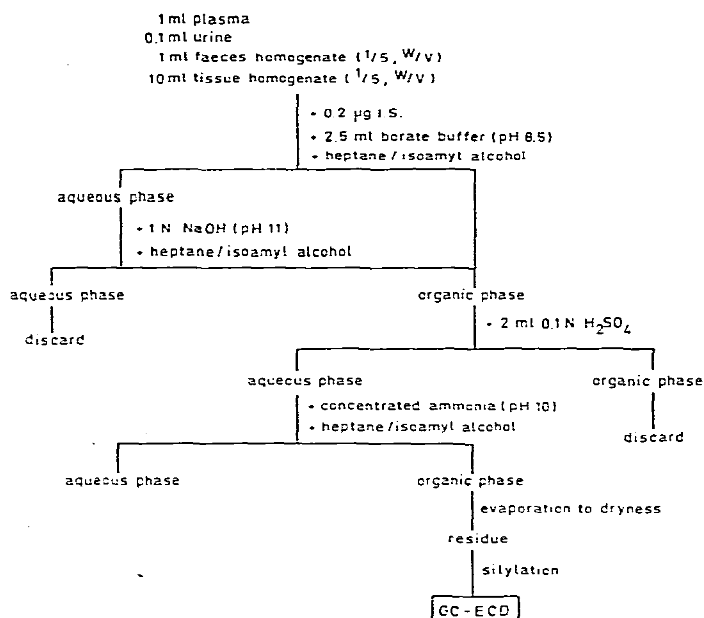


Fig. 2. Extraction scheme for lorcainide and its principal metabolites from plasma, urine, faeces and tissues.

the control of the autosampler functions were carried out by a Spectra-Physics Model 4000 data system.

Derivatization

The extraction residues were dissolved in 10 μl of BSA, containing 1% trimethylchlorosilane, and 100 μl of acetonitrile. The tubes were capped tightly, vortex-mixed, and allowed to stand for 10 min in an oil bath at 90°. After cooling, the samples were transferred to 200-μl microvolume vials and placed in the autosampler. Sample volumes of 1 μl were injected and cleaning of the injection system was achieved by alternating sample vials and vials of pure solvent.

Calculations

Results were calculated by determining the peak area ratios of the different compounds, related to the internal standard and comparing these ratios with the appropriate standard curves.

RESULTS

The recoveries of the extraction procedure, the detection limits and the retention times of lorcainide, its metabolites and the internal standard are summarized in Table I. The plots of the standard curves all passed through zero and were linear over the concentration range 0.01—3.0 μg/ml plasma, having correlation coefficients of 0.999 (Table II).

TABLE I

ANALYTICAL DATA FOR THE REPORTED PROCEDURE

Compound		t_R (min)	RRT**	Percentage recovery*** (mean ± S.D., n = 5)	Detection limit (ng/ml plasma)
R 15665*	(II)	1.88	0.57	85 ± 3	10
Lorcainide	(I)	2.53	0.77	81 ± 4	5
R 17251	(V)	3.28	1.00	88 ± 6	—
R 39611*	(III)	4.28	1.30	78 ± 4	10
R 41853*	(IV)	5.87	1.79	69 ± 8	15

*Analyzed as the trimethylsilyl derivatives.
**RRT = relative retention time of the compounds (relative to the internal standard, R 17251).
***Percentage recovery = recovery of the extraction procedure, obtained after analysis of 1 μg of the appropriate compound added to 1 ml of control plasma.

TABLE II

CORRELATION COEFFICIENTS AND MATHEMATICAL EXPRESSIONS OF THE STANDARD CURVES OF LORCAINIDE AND ITS METABOLITES

R 15889	(I)	$y = 5.097x + 0.039$	$r = 0.9997$
R 15665	(II)	$y = 4.455x - 0.085$	$r = 0.9996$
R 39611	(III)	$y = 4.336x - 0.004$	$r = 0.9998$
R 41853	(IV)	$y = 4.413x - 0.094$	$r = 0.9996$

DISCUSSION

Although the extraction of the unaltered drug and its internal standard was rather simple, an additional extraction step at pH 8.5 was necessary for the optimum recovery of the hydroxylated metabolites III and IV. The formerly used extraction step at pH 11, however, was maintained because it led to a higher recovery for the dealkylated metabolite II. Its recovery was further improved by taking a ratio of 95:5 for the heptane—isoamyl mixture instead of the more commonly used mixture ratio of 98.5:1.5. This improvement was also achieved using hexane—diethyl ether (50:50) and toluene—butanol (90:10) mixtures although both these latter mixtures gave a few more extraneous peaks. No interfering peaks were observed using the heptane—isoamyl alcohol (95:5) mixture.

It is clear that for the determination of lorcainide alone, as, for example, after intravenous administration, the extraction method can be simplified as has been described by Jähnchen et al. [9].

As the metabolites examined are rather polar, they are not very suitable for direct gas–liquid chromatographic analysis and therefore they have to be derivatized to obtain reliable results. Both the N-dealkylated and the hydroxylated metabolites were easily derivatized with dimethylformamide dimethylacetal, but the resulting derivatives still showed some adsorption and yielded larger retention times. Trimethylsilyl (TMS) derivatives were chosen

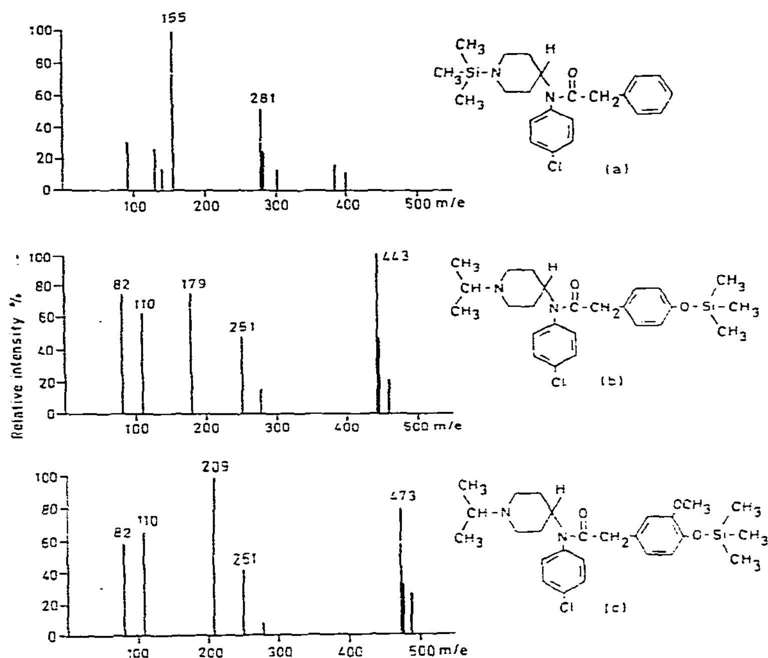


Fig. 3. Mass spectra of the TMS derivatives of compounds II (a), III (b) and IV (c).

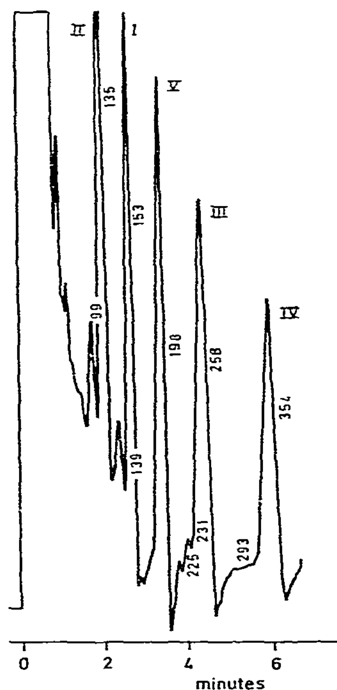


Fig. 4. Chromatogram of a heart (ventriculus cordis) tissue extract from a dog chronically treated with daily oral doses of 10 mg/kg body weight. GLC conditions were as indicated in the text.

because they reacted rapidly, yielding derivatives with much improved column characteristics under the chromatographic conditions described. The O-silylation with BSA occurred instantaneously and quantitatively, the N-silylation yielded at least about 95% after heating the reaction mixture at 90° for 10 min and adding 1% TMCS to the derivatization reagent to catalyze the reaction. Structure identification of the various TMS derivatives was recently confirmed by combined gas chromatography—mass spectrometry and nuclear magnetic resonance spectroscopy [6]. Mass spectra and structures are shown in Fig. 3.

The application of the method to the assay of several hundred specimens of biological origin demonstrated its suitability; other metabolites were easily detected when present and did not interfere with lorcainide or its most important metabolites. The gas chromatographic column has proved to be extremely stable under the conditions used.

APPLICATIONS

The method described has been used successfully for over one year in pharmacokinetic studies on the fate of lorcainide in healthy volunteers and patients as well as in animals [8]. Typical plasma levels from a patient receiving chronic oral administration are presented in Table III. The patient received five consecutive doses of 100 mg at 0, 12, 24, 36 and 48 h and the time course of the plasma levels was then followed up to 32 h after the last dose. After ten days, the same schedule was repeated with doses of 150 mg of lorcainide hydrochloride.

The method has also proved to be valuable in tissue distribution studies of dogs receiving 0.625, 2.5 and 10 mg/kg body weight [7]. A chromatogram of a heart tissue extract, showing all three metabolites, is represented in Fig. 4.

TABLE III

PLASMA LEVELS FOLLOWING CHRONIC ADMINISTRATION OF 100 mg (A) AND 150 mg (B) OF LORCAINIDE HYDROCHLORIDE

Time (h)	Lorcainide hydrochloride (μg/ml plasma)	
	A	B
0	0.214	0.481
2	0.234	0.627
4	0.406	0.873
6	0.285	0.602
8	0.285	0.562
10	0.296	0.437
12	0.215	0.429
24	0.161	0.393
26	0.192	0.421
28	0.171	0.321
30	0.125	0.380
32	0.155	0.294

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