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Note

High-performance liquid chromatographic method for the determination of *p*-hydroxylated and hydroxymethylated metabolites of mexiletine in human serum

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Mexiletine hydrochloride (III, Fig. 1, Mexitil®) is an antiarrhythmic drug that has been shown to be effective for the suppression of ventricular arrhythmias [1–4]. Mexiletine is eliminated primarily by metabolism which takes place largely in the liver [5]. Eight metabolites have been isolated and identified in human urine [6,7], two of them [*p*-hydroxymexiletine (I) and hydroxymethylmexiletine (II)] being considered to be major metabolites (Fig. 1). The determination of these metabolites in urine has been performed by gas chromatography [6,7] and high-performance liquid chromatography (HPLC) with fluorescence detection [8]. Farid and White [9] reported a selective and sensitive gradient HPLC method for the simultaneous determination of mexiletine and the two unconjugated metabolites in human serum.

This paper describes a simple, sensitive and isocratic HPLC method using UV detection for the determination of two major unconjugated metabolites in human serum.

EXPERIMENTAL

Reagents and chemicals

p-Hydroxymexiletine and hydroxymethylmexiletine were obtained from Boehringer Ingelheim (Vienna, Austria). Demoxepan as internal standard was obtained from Hoffmann-La Roche (Basle, Switzerland). Acetonitrile, diisopropyl ether and dichloromethane were purchased from E. Merck (Darmstadt, F.R.G.). All other reagents were of analytical-reagent grade.

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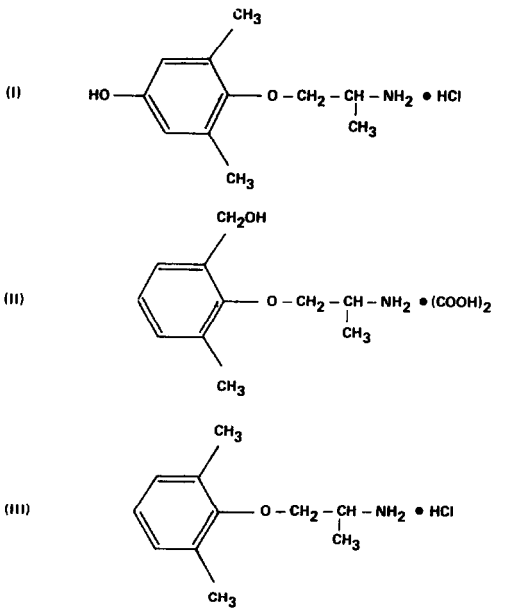

Fig. 1. Structures of: (I) *p*-hydroxymexiletine, 1-(2,6-dimethyl-4-hydroxyphenoxy)-2-aminopropane; (II) hydroxymethylmexiletine, 1-(2-hydroxymethyl-6-methylphenoxy)-2-aminopropane; and (III) mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane.

Preparation of standard solutions

Standard solutions were prepared by dissolving I, II and the internal standard in methanol at concentrations of 1 mg/ml. The standard solutions were stored at 4°C and remained stable for at least six months. Calibration standards were prepared by adding the appropriate diluted standard solutions to pooled serum.

Chromatography

The chromatographic separation was performed isocratically with a single pump (Model 8500; Varian, Walnut Creek, CA, U.S.A.). Injections were made with a syringe loading injector with a 50-μl loop (Model 7125; Rheodyne, Cotati, CA, U.S.A.). The peaks were detected with a Varichrom UV detector (Varian) at 214 nm. A 100 mm × 4.6 mm I.D. C_8 RAC II column packed with particles of average diameter 5 μm (Whatman, Maidstone, U.K.) was used. The column was protected with a guard column (30 mm × 4.6 mm I.D.) packed with 5-μm C_8 particles (Brownlee Labs., Santa Clara, CA, U.S.A.). The mobile phase was prepared by mixing 280 ml of acetonitrile, 714 ml of water and 6 ml of 0.5 *M* potassium dihydrogenphosphate. The mobile phase flow-rate was 70 ml/h. The peaks were recorded with a CDS 111 integrator and recorder (Model 9176; Varian) with a chart speed of 0.25 cm/min. All analyses were performed at ambient temperature.

Sample preparation

A 1-ml volume of serum was transferred into a 15-ml glass centrifuge tube to which 50 μl (250 ng) of the internal standard solution and 0.1 ml of borate buffer

(pH 13) were added. The serum was extracted with a mixture of 4 ml of diisopropyl ether and 1 ml of dichloromethane for 5 min. The sample was frozen at −14°C in a methanol bath. The organic phase was transferred into another tube. Next, 5 ml of diisopropyl ether were added to serum and the mixture was extracted for 5 min. The sample was frozen again in a methanol bath and the organic phase was added to the previously separated one. The mixed extracts were evaporated to dryness in a stream of nitrogen, the residue was reconstituted in 100 μl of mobile phase and 50 μl were injected on to the column.

Calibration graphs

Calibration graphs were constructed with 1 ml of blank serum containing 50, 100, 200, 500 and 1000 ng of both *p*-hydroxymexiletine and hydroxymethylmexiletine and 250 ng of demoxepam as internal standard. These samples were extracted by the method described above. Least-squares linear regression lines of the peak height of each compound versus concentration were calculated. The slope and intercept of each line were used to calculate *p*-hydroxymexiletine and hydroxymethylmexiletine concentrations in unknown samples.

RESULTS AND DISCUSSION

Trials of the simultaneous determination of mexiletine and its unconjugated metabolites *p*-hydroxymexiletine and hydroxymethylmexiletine under isocratic conditions on a reversed-phase column showed that the metabolites exhibit retention times of 1.20 and 1.36 min, respectively, and the parent drug 6.10 min [10]. These short retention times cause both metabolites to be covered by the

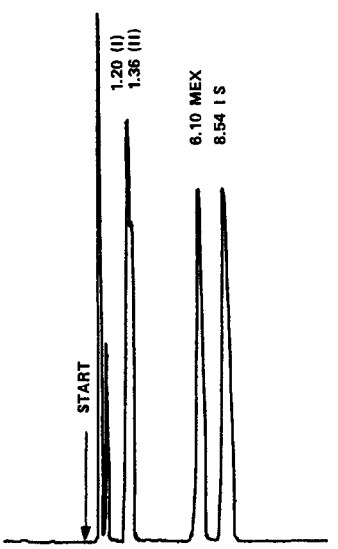

Fig. 2. Chromatogram obtained from a standard sample with 1 mg/l each of I and II and 0.25 mg/l internal standard (I.S.) added. Conditions for determination of mexiletine: mobile phase, acetonitrile–water–0.5 *M* potassium dihydrogenphosphate (380:610:10); flow-rate, 70 ml/h; detection, 214 nm.

biological background from the serum (Fig. 2). The large difference between the retention times of the drug and its metabolites arises because both of the latter are polar owing to the existence of a hydroxy group, which is lacking in mexiletine (Fig. 1). For these reasons, it was impossible to determine the parent drug and its metabolites simultaneously under isocratic conditions.

In order to find suitable conditions allowing the determination and quantitative recovery of *p*-hydroxymexiletine and hydroxymethylmexiletine, a number of mixtures with different proportions of two of three components, i.e., water and

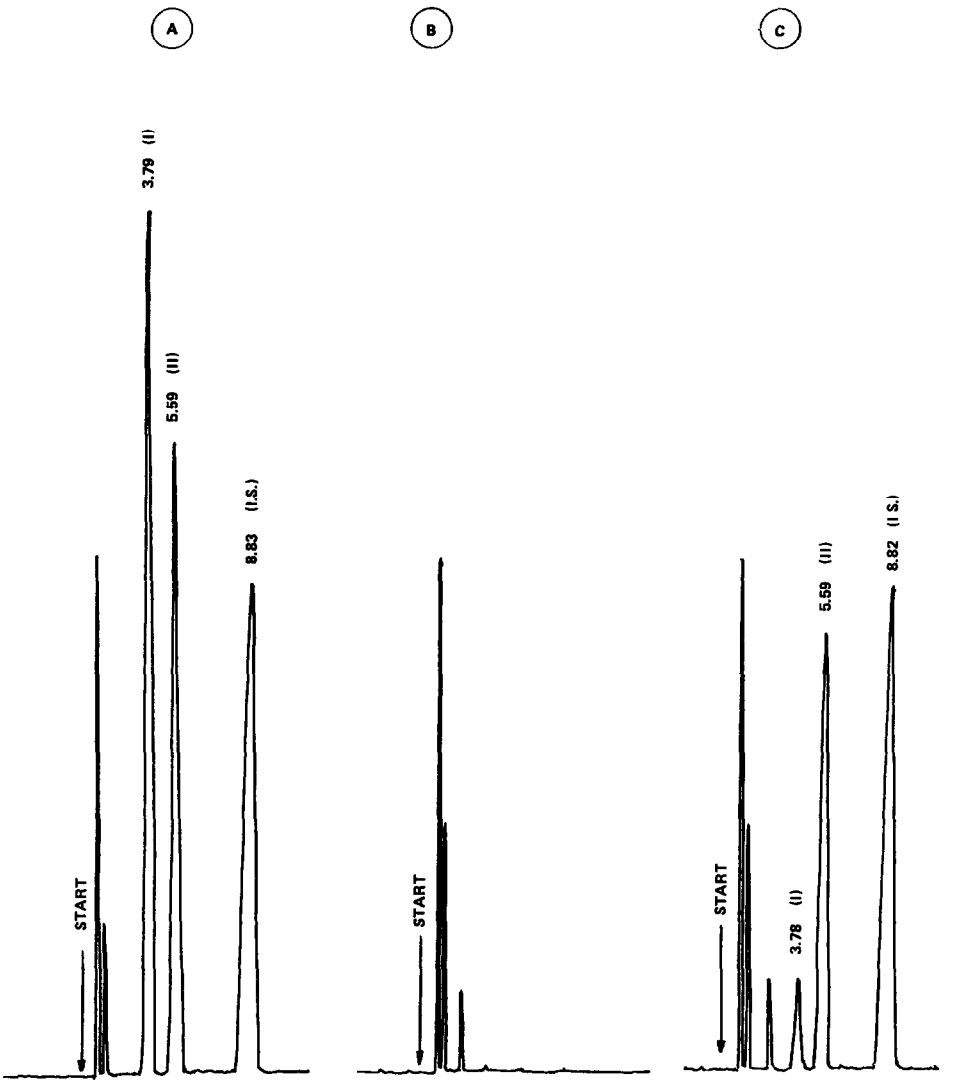

Fig. 3. Chromatograms obtained from: (A) standard sample containing 1 mg/l each of I and II and 0.25 mg/l I.S; (B) drug-free serum; (C) patient's serum 4 h following a single 200-mg oral dose of mexiletine containing 0.105 mg/l I and 0.652 mg/l II. Conditions: mobile phase, acetonitrile–water–0.5 *M* potassium dihydrogenphosphate (280:714:6) ; flow-rate, 70 ml/h; detection, 214 nm; double extraction with diisopropyl ether–dichloromethane (80:20) and diisopropyl ether.

0.5 *M* phosphate buffer, were prepared. Using these mixtures as the mobile phase the retention time and absorbance response for the investigated metabolites were measured. The optimal resolution was obtained using a mobile phase containing 280 ml of acetonitrile, 714 ml of water and 6 ml of 0.5 *M* phosphate buffer.

Considerable efforts were made to find an appropriate internal standard and the best was found to be demoxepam. Numerous experiments were carried out with this internal standard and the results indicated that in all instances the peaks were well separated and the recovery was high.

As diisopropyl ether appeared to be a very good extraction reagent for the parent drug, it was subsequently used for the extraction of *p*-hydroxymexiletine, hydroxymethylmexiletine and the internal standard. Whereas the recovery for the first metabolite (I) was about 82% (average of five analyses), for hydroxymethylmexiletine and the internal standard it was low, being about 50 and 66%, respectively (average of five analyses). In order extraction to increase the recovery of these compounds, diisopropyl ether–dichloromethane in various ratios was used as the extraction mixture. It has been found that with a ratio of 80:20 (v/v) the recoveries of II and the internal standard were higher (75 and 81%, respectively) but that of I decreased to 69%. Finally, a double extraction was applied, first using diisopropyl ether–dichloromethane (80:20) and then with diisopropyl ether alone. Using this procedure the recoveries were 96% for *p*-hydroxymexiletine, 90% for hydroxymethylmexiletine and 95% for demoxepam.

Typical chromatograms obtained by applying the procedure to a patient's serum are shown in Fig. 3. The peaks of I, II and demoxepam were well separated from the endogenous peaks, and their retention times were approximately 3.79, 5.59 and 8.83 min, respectively. Each of these peaks was sharp and symmetrical.

The calibration graphs for the determination of *p*-hydroxymexiletine and hydroxymethylmexiletine in serum in the range 0.01–1.0 mg/l showed excellent linearity ($r=0.999$). Table I shows the results of precision and accuracy tests.

TABLE I

PRECISION AND ACCURACY OF THE DETERMINATION OF *p*-HYDROXYMEXILETINE (I) AND HYDROXYMETHYLMEXILETINE (II) IN SERUM ($n=5$)

Compound	Concentration (mg/l)	Peak-height ratio (mean ± S.D.)	Accuracy (mean ± S.D.) (mg/l)	Coefficient of variation (%)
I	0.05	0.085 ± 0.005	0.052 ± 0.002	6.3
	0.10	0.180 ± 0.010	0.110 ± 0.005	5.6
	0.20	0.367 ± 0.013	0.196 ± 0.007	3.7
	0.50	0.927 ± 0.020	0.493 ± 0.011	2.2
	1.00	1.880 ± 0.028	1.010 ± 0.014	1.5
II	0.05	0.072 ± 0.006	0.048 ± 0.004	6.0
	0.10	0.138 ± 0.008	0.094 ± 0.008	8.5
	0.20	0.255 ± 0.015	0.205 ± 0.011	5.8
	0.50	0.675 ± 0.017	0.489 ± 0.013	2.6
	1.00	1.320 ± 0.028	0.979 ± 0.020	2.1

The limit of quantitation for both metabolites in human serum was 0.05 mg/l.

Drugs that were frequently used together with mexiletine in patients with ventricular arrhythmia and myocardial infarction (disopyramide, quinidine sulphate, nifedipine, propranolol, metoprolol, procainamide, amiodarone and verapamil) were examined for interferences. Other drugs such diazepam, nitrazepam, phenytoin and theophylline were also tested. These drugs were added to drug-free serum and assayed in order to detect whether they interfered with the chromatograms of *p*-hydroxymexiletine and hydroxymethylmexiletine. No interferences were found.

CONCLUSION

The proposed HPLC method using a C_8 RAC II column under isocratic conditions for the determination of the mexiletine metabolites *p*-hydroxymexiletine and hydroxymethylmexiletine in clinical serum samples is simple and rapid with a high recovery of investigated compounds, and can be recommended for routine patient monitoring and pharmacokinetic studies.

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