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Note

Rapid high-performance liquid chromatographic method for the quantification of mexiletine and its metabolites in serum

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Mexiletine, 1-methyl-2-(2,6-xylyloxy)ethylamine hydrochloride (Mexitil®), has been shown to suppress markedly ventricular rhythm disorders [1–6]. Effective serum levels of mexiletine range from ca. 0.5 to 2.0 μg/ml [2–4,7,8]. Although the antiarrhythmic efficacy of mexiletine is not correlated directly with its serum level [3,4,7,8], the determination of the drug is recommended when adverse effects occur, when drug therapy is ineffective in order to differentiate failure of therapy from suboptimal dosing, in patients with cirrhosis of the liver, or in order to check a patient's compliance.

Gas chromatographic (GC) [9–12] and high-performance liquid chromatographic (HPLC) methods [13–23] have been widely used for the determination of serum or plasma levels of mexiletine. GC methods are sensitive and specific but rather time-consuming, and require equipment that is not routinely available in clinical laboratories. Most HPLC methods need either fluorescence detection [13,16,19,20,23] or derivatization [14,16,18,19,20,23], and only some use UV detection with no need for either [15,17,21,22].

The aim of this study was to develop an HPLC assay for the simultaneous determination of mexiletine hydrochloride and mexiletine metabolites. The method had to be rapid, simple and accurate in the therapeutic range and inexpensive, to allow for easy monitoring of the drug in clinical practice.

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EXPERIMENTAL

Chemicals and reagents

Mexiletine, mexiletine metabolite 1 (KOE 2259-OX) [1-(2-hydroxymethyl-6-methylphenoxy)-2-aminopropane oxalate], metabolite 2 (KOE 2127-CL) [1-(2,6-dimethyl-4-hydroxy)-2-amino-propane hydrochloride] and the internal standard (KOE 768-CL) [1-(2,4-dimethylphenoxy)-2-aminopropane hydrochloride] were provided by Boehringer Ingelheim (Ingelheim, F.R.G.); acetonitrile, hydrochloric acid, triethylamine, dichloromethane and sodium hydroxide were obtained from Merck (Darmstadt, F.R.G.); 1-octanesulphonic acid (PIC B-8 low-UV reagent) and dibutylamine phosphate (PIC D-4 reagent) came from Waters Assoc. (Eschborn, F.R.G.); butylamine was obtained from Fluka (Buchs, Switzerland).

Instruments

The chromatographic system consisted of a Model 721 system controller, a 510 HPLC pump, a Wisp 710B injector block, a Lambda-Max Model 481 variable-wavelength detector and a Data Module Model 730, all from Waters Assoc.

Chromatographic conditions

The analysis was performed using a Shandon Hypersil CPS (CN) reversed-phase column (250 mm × 4.6 mm I.D., particle size 5 μm) (Grom, Herrenberg, F.R.G.) at room temperature. The mobile phase consisted of 95% A (973.5 ml of water, 25 ml of PIC B-8 low-UV reagent, 1 ml of butylamine, 0.5 ml of PIC D-4 reagent) and 5% B (acetonitrile). The flow-rate was 2.0 ml/min. The column effluent was monitored at 215 nm, using a detector range of 0.02 a.u.f.s. and a chart speed of 0.4 cm/min. The injection volume was 200 μl.

Standards

Two stock standard solutions were prepared, containing 20 μg/ml each of mexiletine hydrochloride, mexiletine metabolites and internal standard (I.S.) in 0.01 *M* hydrochloric acid. The solutions were stable for at least two months if stored at 4 °C. A standard working solution was obtained by combining aliquots of the stock solutions and diluting with hydrochloric acid in concentration of 2.0 μg/ml for either mexiletine hydrochloride, mexiletine metabolites or the I.S.

Sample collections

Serum was obtained by centrifugation at 900 *g* for 10 min. Twenty-two serum samples from normal volunteers (twelve male, ten female) were pooled (normal pool). Twenty-four serum samples from patients (sixteen male, eight female) treated with cardiovascular drugs, such as acebutolol, atenolol, betaxolol, metoprolol, pindolol, sotalol, digoxin, nifedipine, verapamil, amiodarone, flecainide, propafenone, quinidine, phenprocoumone, acenocoumarol, acetyl-

salicylic acid, dipyridamol, sulfinpyrazone, spironolactone, furosemide, hydrochlorothiazide and triamterene, were pooled in four pools (patient pools). Twenty serum samples from twenty patients (seventeen male, three female) treated with mexiletine (360–1440 mg per day) were analysed individually.

Extraction procedure

A 1.75-ml volume of dichloromethane, 0.25 ml of triethylamine, 1.0 ml of serum, 100 µl of 1 *M* sodium hydroxide and 100 µl of the I.S. stock solution were placed in a glass centrifuge tube (Kästner, Tübingen, F.R.G.). The tube was closed with a stopper (Sarstedt, Nürmbrecht, F.R.G.), shaken (by slow rotation) for 20 min and centrifuged at 3600 *g* for 10 min. Subsequently the serum phase was discarded. Then 1.0 ml of the dichloromethane–triethylamine phase was transferred to a clean glass tube and evaporated to dryness at room temperature under a stream of nitrogen. The residue was redissolved in 500 µl of 0.01 *M* hydrochloric acid.

Linearity test

The linearity of the chromatographic procedure was tested for mexiletine hydrochloride, mexiletine metabolites and the I.S. by analysing six standard solutions with the concentrations 4.0, 2.0, 1.0, 0.5, 0.25 and 0.125 µg/ml for each of the components. The linearity test of the entire procedure was done with the same standard solutions. The amount of each standard required to reach concentrations in the range 0.125–4.0 µg/ml was introduced into a glass centrifuge tube, and 1.0 ml of the normal pool serum was added. Subsequently the spiked serum samples were extracted and chromatographed.

Recovery

The recovery was determined by comparing the peak areas of mexiletine hydrochloride, mexiletine metabolites and the I.S. obtained by analysing a spiked serum sample (100 µl of each stock solution standard plus 1.0 ml of the normal pool) with the peak areas obtained by direct injection of the standard working solution (2.0 µg/ml each of mexiletine hydrochloride, mexiletine metabolites and the I.S.).

Quantification

The determination of the concentration of mexiletine hydrochloride and its metabolites in the serum was based on calibration graphs obtained from standard solutions (0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 µg/ml). The lower limit of quantification was 0.05 µg/ml for mexiletine and 0.02 µg/ml for its metabolites. Since the calibration graph was linear and the calibration was stable for three months, the calculation was performed with the 2.0 µg/ml of each of the standard compounds, using the following formula:

concentration of mexiletine in sample (μg/ml)

$$= \frac{\text{peak area of mexiletine (or metabolites) in sample}}{\text{peak area of external standard}} \times \frac{100}{\text{recovery of I.S. in sample}} \times 2$$

The recoveries of mexiletine, its metabolites and the I.S. were shown to be the same within ca. 5%.

RESULTS

For the standards, as well as for the entire procedure, the linearity test showed a linear relationship between concentration and peak area for the total range tested. The recoveries (mean ± S.D.) were 98.5 ± 2.3% for mexiletine hydrochloride, 99.9 ± 8.2% for mexiletine metabolite 1, 96.7 ± 6.6% for mexiletine metabolite 2 and 95.8 ± 3.6% for the I.S. (mean ± S.D. of ten determinations; the technical error of the apparatus was less than 1% for mexiletine, mexiletine metabolites and the I.S.).

TABLE I

SERUM LEVELS OF MEXILETINE HYDROCHLORIDE AND ITS METABOLITES IN PATIENTS TREATED CHRONICALLY WITH MEXILETINE

Patient No.	Oral dose of mexiletine hydrochloride per day (mg)	Serum level before mexiletine administration (μg/ml)		
		Mexiletine hydrochloride	Metabolite 1	Metabolite 2
1	360[a]	1.00	0.10	0.04
2	400[b]	0.89	0.16	0.30
3	400[b]	0.74	<0.02	0.29
4	400[b]	0.74	1.19	0.04
5	600[b]	0.20	0.72	0.06
6	600[b]	0.96	0.29	0.28
7	600[b]	0.55	0.44	0.46
8	600[b]	1.33	<0.02	<0.02
9	600[b]	1.23	0.60	0.14
10	720[a]	1.01	0.39	<0.02
11	720[a]	3.28	0.08	<0.02
12	720[a]	1.71	0.29	0.08
13	720[a]	1.19	0.56	<0.02
14	720[a]	2.20	0.38	0.39
15	800[b]	2.31	0.77	<0.02
16	1080[a]	2.87	0.26	0.48
17	1200[b]	1.94	0.04	0.49
18	1200[b]	2.69	0.16	0.22
19	1200[b]	2.01	0.16	0.72
20	1440[a]	4.83	0.39	0.75

[a]Slow-release formulation.
[b]Instant-release formulation.

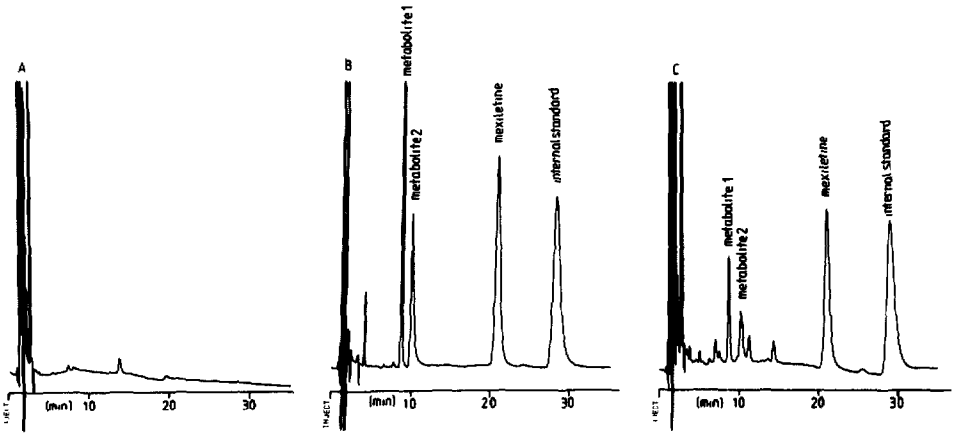

Fig. 1. Chromatograms of a blank from the normal pool (A), of a standard working solution (B) and of the serum of a patient receiving mexiletine (C).

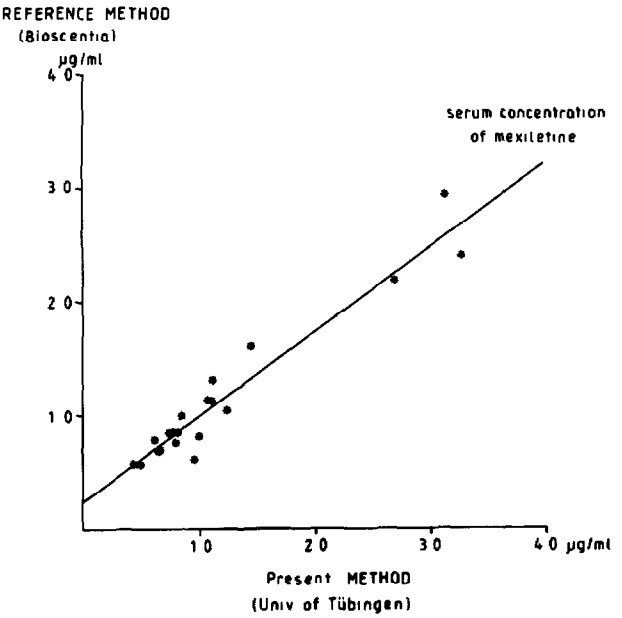

Fig. 2. Comparison of serum concentration of mexiletine hydrochloride determined by the present HPLC method and by the method used by Bioscentia ($r=0.9650$, $n=20$).

We did not observe any cardiovascular substances as interfering peaks in the patients' pools. The individual levels of mexiletine hydrochloride and mexiletine metabolites (level before mexiletine administration) are shown in Table I. A typical chromatogram obtained from the analysis of a serum sample of a patient receiving mexiletine hydrochloride is shown in Fig. 1. A comparison of

the results from twenty serum samples analysed either in the laboratories of Bioscentia (Ingelheim, F.R.G.) (by HPLC with derivatization and fluorescence detection; modification of the methods of Gupta and Lew [20] and Kelly et al. [21]) and in our laboratory (by the present HPLC method) is shown in Fig. 2.

The coefficients of variation ($n=10$) were 2.3% for mexiletine, 8.2% for metabolite 1 and 6.8% for metabolite 2.

DISCUSSION

The majority of HPLC methods allows the determination of mexiletine only [13–15,18–23] or the determination of mexiletine enantiomers [18,23]. The HPLC method of Farid and White [16] allows the additional determination of the mexiletine metabolites hydroxymethylmexiletine and *p*-hydroxymexiletine, and that of Filipek et al. [17] the determination of the same two metabolites, but not the simultaneous determination of mexiletine. Recoveries of mexiletine and the I.S. and the limit of detection of mexiletine as given in the literature are listed in Table II. Recovery of hydroxymethylmexiletine was 85% [16] and 90% [17] and of *p*-hydroxymexiletine 88% [16] and 96% [17]. The limit of detection was 0.005 [16] or 0.05 μg/ml [17].

The present HPLC method for the simultaneous assay of mexiletine and mexiletine metabolite serum levels yields a nearly complete recovery of mexiletine, its metabolites and the I.S. from serum and a sufficiently low detection limit using UV detection at 215 nm with no need for fluorescence detection or

TABLE II

RECOVERY OF MEXILETINE AND INTERNAL STANDARD AND LIMIT OF DETECTION OF MEXILETINE

Recovery (%)		Limit of detection (μg/ml)	Reference
Mexiletine	I.S.		
–	–	0.05	13
46–69	49–74	0.01	14
87	101	0.1	15
81	–	0.005	16
>90	–	0.05	18
>90	–	0.001	19
75–80	–	–	20
>90	>90	0.2	21
68–78	72–87	0.05	22
–	–	0.005	23
98	96	0.05	Present method

derivatization. No cardiovascular substance or endogenous substance was observed as an interfering peak, and an excellent correlation with an established HPLC method was demonstrated.

In conclusion, this HPLC method is suitable for the clinical management of patients and for research into the effect of mexiletine metabolites on side-effects and efficacy. Measurements can be performed in every laboratory possessing an HPLC system with a variable-wavelength UV detector without the need for additional equipment.

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