

Improved Liquid-Chromatographic Determination of Mexiletine, an Antiarrhythmic Drug, in Plasma

Walter Mastropaolo^{2,3} David R. Holmes,² Michael J. Osborn,² Julianne Rooke,² and Thomas P. Moyer^{1,4}

In this improved reversed-phase liquid-chromatographic procedure for determination of mexiletine in plasma, mexiletine and an internal standard, chlorodisopyramide, are extracted with methylene chloride from 0.5 mL of serum or plasma; the extract is then concentrated and injected onto a C₁₈ chromatographic column. Mexiletine in the column effluent is detected by monitoring absorbance at 210 nm. It is quantified by use of mexiletine-internal standard peak-height ratios. The relation between this ratio and mexiletine concentration is linear from 0.1 to 5.0 mg/L. The lower limit of detection is about 50 µg/L. At a mexiletine concentration of 2.0 mg/L in serum, intrarun precision (CV) is 2.9% and inter-run precision is 5.9%; at 0.5 mg/L, these CVs are 5.7% and 9.6%, respectively. Analytical recovery of added mexiletine in serum is 68-88%. Therapeutic concentrations of some commonly administered drugs in patients' specimens did not interfere. In serum from 38 patients receiving mexiletine for cardiac arrhythmia, concentrations measured by this method correlated with therapeutic efficacy.

Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane, is a class I antiarrhythmic agent for treatment of ventricular arrhythmias. It currently is undergoing trials in the United States. A primary amine similar in chemical structure and electrophysiological action to lidocaine, it acts by depressing the maximal rate of depolarization in atrial, ventricular, and Purkinje cells (1-3). Unlike lidocaine, mexiletine can be administered either orally or intravenously. It appears to be effective in 60 to 80% of patients with ventricular arrhythmias refractory to treatment by other drugs (4-6). Its optimal concentration range in serum has been defined as 0.75-2.0 mg/L (7, 8); therefore its use will require careful monitoring to enhance efficacy and avoid toxicity.

Several gas-chromatographic methods for measurement of mexiletine in serum have been reported (9-16). Of the two previously published "high-performance" liquid-chromatographic procedures for mexiletine, one (17) requires derivatization of the sample, multiple sample extractions, and a chromatographic enrichment system. Such complexity makes this assay impractical for most clinical laboratories. The other such procedure (18) is too insensitive, imprecise, and susceptible to interferences for routine use.

Here we report our liquid-chromatographic procedure for sensitive, specific, and precise determination of mexiletine. The necessary pump, column, and detector are available in most clinical laboratories doing liquid-chromatographic analyses.

Materials and Methods

Apparatus. We determined the ultraviolet spectrum of mexiletine with a Hitachi Model 200 dual-beam scanning spectrophotometer (supplied through Perkin-Elmer Corp., Coleman Instrument Div., Oak Brook, IL 60521).

For chromatography, samples were injected through a Model 7125 injector (Rheodyne, Cotati, CA 94928) onto a 30 cm × 3.9 mm µBondapak C₁₈ octadecylsilane column packed with particles 10 µm in diameter (Waters Associates, Inc., Milford, MA 01757). Absorbance of the effluent from the column at 210 nm was measured with a variable-wavelength spectrophotometer (Model LC 55; Perkin-Elmer Corp.) equipped with a flow cell having a 1-cm pathlength, interfaced with a Model 3390 recorder/integrator (Hewlett-Packard Co., Avondale, PA 19311). The pump for the mobile phase was a Model 110A (Beckman Instruments, Inc., Irvine, CA 92713).

Reagents and standards. The stock solution of mexiletine contained 1.0 g of the free base per liter. It was prepared by dissolving 12.0 mg of mexiletine HCl (a gift from Boehringer Ingelheim, Ltd., Richfield, CT 06877) in 10.0 mL of water. Working standard, 1.0 mg/L, was prepared by diluting 0.25 mL of stock mexiletine to a total volume of 250 mL with sodium carbonate buffer (0.1 mol/L, pH 10.4). Stock and working mexiletine solutions are stable for at least six months at 4 °C. Serum controls, 0.5 and 2.0 mg/L, were prepared by making appropriate dilutions of stock mexiletine in bovine serum (Pel-Freez Biologicals, Rogers, AR 72756). Aliquots (0.5 mL) of control sera, pipetted into disposable 16 × 125 mm glass tubes and stored at -20 °C, are stable for at least six months.

Stock chlorodisopyramide (a gift from G. D. Searle Co., Skokie, IL 60077), 1.0 g/L, was prepared by dissolving 10 mg of free base in 10.0 mL of methanol (stable for at least two years at -20 °C). A working solution of internal standard (40 mg/L) was prepared by diluting 0.40 mL of stock chlorodisopyramide in water to a total volume of 10 mL. Such a solution is stable for at least six months at 4 °C.

Methylene chloride and acetonitrile were glass distilled (Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442). All other chemicals were reagent grade.

Procedure. Pipet 0.5 mL of 1.0 mg/L working standard, serum or plasma sample, or control serum into a 16 × 125 mm disposable glass tube. To each tube, add 100 µL of the 40 mg/L solution of chlorodisopyramide. To all tubes except those containing working standard, add 100 µL of a 0.5 mol/L sodium carbonate solution, pH 11.3. The final pH of serum or plasma sample should now be 10.4 (this pH is critical to reproducible extraction). Mix the contents of each tube, add 7.0 mL of methylene chloride, and shake the mixture for 1.0 min. Centrifuge at 2000 × g for 2 to 3 min to separate the phases, then transfer 5 mL of the methylene chloride (lower) phase to a 15-mL conical glass tube, add 50 µL of 0.1 mol/L HCl in methanol, and mix. Evaporate the solvent under a stream of nitrogen (this may be done at

¹ Department of Laboratory Medicine and ² Division of Cardiovascular Diseases and Internal Medicine, Mayo Clinic, Rochester, MN 55905.

³ Present address: Clinical Laboratory, Memorial Hospital, New Albany, IN 47150.

⁴ To whom correspondence should be addressed.

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40 °C to speed evaporation). Reconstitute the residue by adding 100 μ L of the mobile phase—a mixture of potassium phosphate (0.1 mol/L, pH 4.0) and acetonitrile (75/25 by vol)—and vortex-mixing for 30 s. Chromatograph an aliquot of the reconstituted extract at a flow rate of 3.0 mL/min.

Quantify the mexiletine in samples or controls by dividing the peak-height ratio of mexiletine to internal standard by the mean of the same ratio for duplicate determinations of standards.

Studies with patients' samples. To assess the performance of the procedure under routine conditions, we determined plasma mexiletine concentrations in specimens from 38 patients who were receiving mexiletine for the control of ventricular arrhythmias. Samples for determining trough values were obtained from the patient just before a scheduled dose.

Results

Figure 1 depicts typical chromatograms for the controls and patients' specimens. The mexiletine peak corresponding to a concentration of 0.5 mg/L, the lower limit of the therapeutic range, considerably exceeds background. All chromatograms were free of endogenous interferences. The retention time for mexiletine is about 2.7 min. Total chromatography time for the assay is less than 8 min per run.

Peak-height ratios of mexiletine to internal standard are linearly related to mexiletine concentration in both human and bovine serum over the range 0.1 to 5.0 mg/L (Figure 2). The therapeutic range for mexiletine (0.75–2.0 mg/L) is well within the linear portion of this standard curve. Because the standard curves based on human and bovine serum are equivalent, we prefer to use bovine serum in controls, for economy and to avoid risk of hepatitis.

We determined analytical recovery of mexiletine and internal standard from serum by comparing the peak heights of extracted samples with those of equivalent

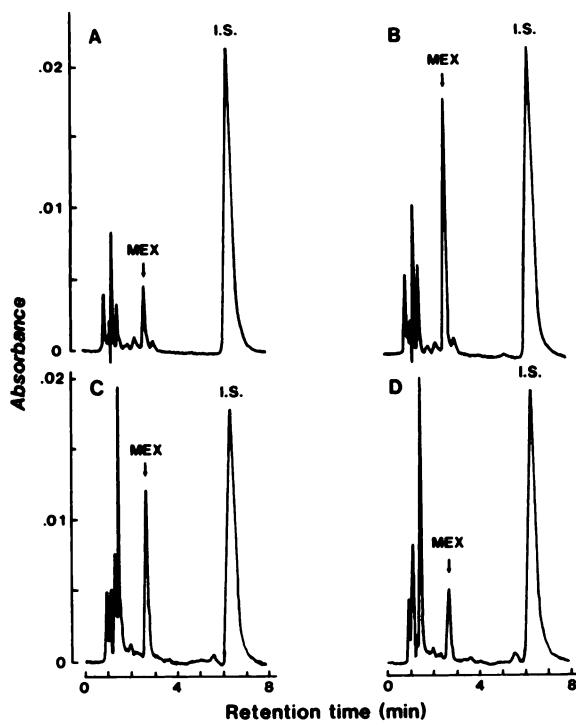


Fig. 1. Liquid chromatograms of bovine serum controls containing 0.5 mg (A) or 2.0 mg (B) of mexiletine (MEX) per liter, and of patients' plasma samples containing 1.74 mg (C) or 0.71 mg (D) of mexiletine per liter

I.S., internal standard (chlorodisopyramide)

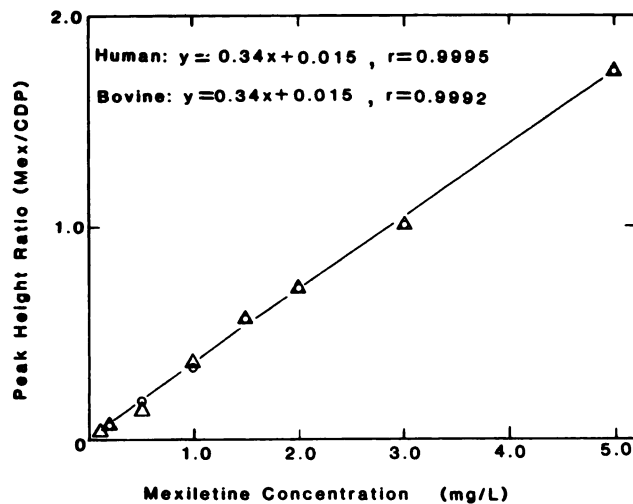


Fig. 2. Standard curves for mexiletine in human (O) and bovine (Δ) serum

amounts of drug and internal standard dissolved in mobile phase and chromatographed directly. Results, corrected for sampling volume, ranged from 68% to 78% for mexiletine, and from 72% to 87% for chlorodisopyramide ($n = 8$) (Table 1). We considered these recoveries adequate. Any further increase in recovery produced by an additional extraction would not improve the sensitivity of the procedure significantly.

Precision was determined at mexiletine concentrations of 0.5 and 2.0 mg/L (Table 2). Intrarun precision was determined by analyzing five samples of the same specimen. Inter-run precision was determined by analyzing 18 samples from a control pool over a period of more than seven months.

We evaluated the possibility of interference by other drugs by adding 2 mg of mexiletine per liter to plasma samples containing known concentrations of any one of various drugs (Table 3). Because these samples were from patients actually taking these drugs, interferences from drug metabolites, if any, would also be detected. Neither this study nor analyses of more than 50 clinical specimens revealed any drug interfering with this procedure.

As shown in Figure 3, 82% of the patients studied had their arrhythmia controlled by mexiletine without side effects. In 87% of these controlled patients, the mexiletine concentrations in plasma were 0.75 to 2.00 mg/L. In two of the three patients showing symptoms of toxicity, mexiletine concentrations exceeded 2.0 mg/L. The dosage was de-

Table 1. Analytical Recovery of Mexiletine and Chlorodisopyramide from Serum

| Concn added, mg/L | Recovery, % Mean (range) |
|---------------------------|--------------------------|
| <i>Mexiletine</i> | |
| 0.5 | 75.7 (73.7–78.3) |
| 2.0 | 71.0 (67.9–73.0) |
| <i>Chlorodisopyramide</i> | |
| 8.0 | 78.6 (71.7–87.5) |

Table 2. Precision of the Present Method

| Mexiletine, mg/L | Intrarun ($n = 5$) | | | Inter-run ($n = 18$) | | |
|------------------|----------------------|-------|-------|------------------------|-------|-------|
| | Mean | SD | CV, % | Mean | SD | CV, % |
| 0.5 | 0.51 | 0.028 | 5.7 | 0.50 | 0.048 | 9.6 |
| 2.0 | 2.06 | 0.060 | 2.9 | 2.09 | 0.11 | 5.4 |

Table 3. Drugs That Do Not Interfere with the Mexiletine Assay

| Drug | Drug concn, mg/L | |
|----------------------|-------------------|---------------------|
| | Therapeutic range | Measured in plasma* |
| Procainamide | 4-10 | 14 |
| N-Acetylprocainamide | 10-30 | 14 |
| Quinidine | 2-5 | 4.2 |
| Lidocaine | 2-5 | 10.1 |
| Disopyramide | 2-4.5 | 4.6 |
| Primidone | 9-12 | 15.3 |
| Phenytoin | 10-20 | 14.6 |
| Carbamazepine | 2-10 | 11.4 |
| Phenobarbital | 20-40 | 41.9 |
| Thioridazine | 0.05-0.15 | 2.89 |
| Diazepam | 0.32-1.80 | 0.55 |
| Nordiazepam | 0.32-1.80 | 3.34 |
| Acetaminophen | 10-20 | 50.6 |
| Salicylate | 10-200 | 25 |
| Theophylline | 10-20 | 24.7 |

* As determined by routine analysis of clinical specimens by immunoassay or liquid chromatography. These concentrations do not interfere.

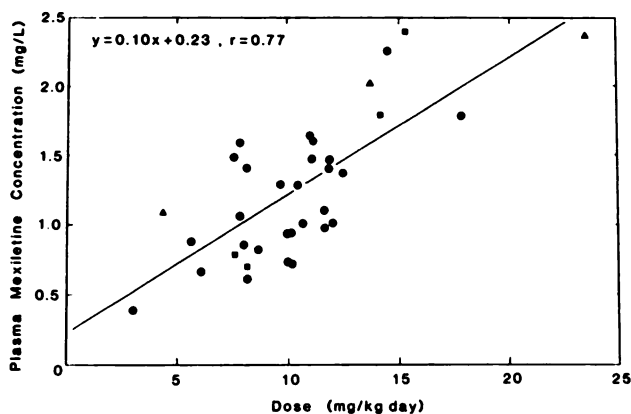


Fig. 3. "Trough" concentrations of mexiletine in plasma from patients as a function of dose

Patients with arrhythmias controlled or partly controlled by mexiletine (●), patients with arrhythmias not controlled by mexiletine (■), patients with symptoms attributed to mexiletine toxicity (▲)

creased for one patient of the group shown in Figure 3, which resulted in a decrease in mexiletine concentration and a cessation of toxic symptoms. Of four patients who were unresponsive to mexiletine, two had drug concentrations of <0.8 mg/L in their plasma. Two uncontrolled patients with high mexiletine concentrations were refractory at a lower mexiletine dosage; their dosage had been increased in an attempt to control the arrhythmia. As shown in Figure 3, concentrations of mexiletine in plasma correlated with dosage ($r = 0.77$).

Discussion

The absorbance of mexiletine at 260 nm is low, but increases greatly at wavelengths lower than 225 nm. We chose to use the wavelength 210 nm for this procedure, because the small inflection in the spectrum at this wavelength lessens the effect of small differences in wavelength setting on the absorbance measured. Sensitivity at 210 nm is 30- to 40-fold greater than at 261 nm, the wavelength used in a previously published procedure (18). In addition, interferences from procainamide and other drugs are greatly diminished at 210 nm as compared with 260 nm.

The mobile phase is qualitatively the same combination

used in this laboratory for several liquid-chromatographic procedures (19). We adjusted the acetonitrile concentration to optimize the capacity factor and assay time for the mexiletine method.

The structural isomer of mexiletine, 1-(2,4-dimethylphenoxy)-2-aminopropane (Kö 768), was unsatisfactory as an internal standard because of differences in its extraction characteristics. In addition, we find its retention time on C₁₈ columns to be identical to that of disopyramide under various chromatographic conditions, as did Kelly et al. (18). Disopyramide probably will be used in combination with mexiletine for control of arrhythmias (20).

Figure 3 demonstrates the close relationship between dosage and concentration of mexiletine in plasma. A change in dosage of 1 mg/kg of body weight per day will on the average produce a 0.1 mg/L change in concentration. The interpatient variation in this study ($r = 0.77$) is somewhat more than that ($r = 0.95$) reported by Pottage (21) but appreciably less ($r = 0.34$) than reported by Campbell et al. (8). Such variations in serum mexiletine concentration can be related to numerous factors—e.g., liver function, urinary pH, and the influence of other drugs (22, 23)—but renal impairment reportedly does not affect the half-life of mexiletine in plasma (24).

Symptoms of toxicity (including tremor, nystagmus, nausea, vomiting, confusion, bradycardia, and hypotension) are said to be associated with concentrations exceeding 2.0 mg/L in serum (7, 8); we agree.

The mexiletine procedure described in this paper is a practical clinical method that has been in routine use in our laboratory for more than six months in support of a clinical trial of mexiletine.

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