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Note

Determination of mexiletine and its metabolites in serum by liquid chromatography with fluorescence detection

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Mexiletine [1-(2′,6′-dimethylphenoxy)-2-aminopropane] (I), Fig. 1, is a new antiarrhythmic drug. While its structure and electrophysiological properties resemble those of lidocaine, I has the advantages of being effective when given orally and having a longer half-life [1]. The pharmacological properties of I have been reviewed recently [1]. The effective therapeutic concentration of I in blood was reported to be 0.5—2.0 μg/ml [2]. However, side effects were noted in some cases with blood concentrations as low as 0.8 μg/ml and severe side effects with concentrations above 2 μg/ml [3]. Prescott et al. [4] reported that the plasma half-life of I was much longer in patients (12—17 h) than in healthy volunteers (9—10 h), and that the half-life was also affected by urinary pH and coadministration of other drugs. This information suggested that therapeutic drug monitoring of blood levels of I may be of value in some patients.

Earlier studies have shown that the major metabolites of I are hydroxymethyl-mexiletine (II) and *p*-hydroxy-mexiletine (III), Fig. 1, and that I, II, and III were further conjugated to form their corresponding glucuronides and also the aryl sulfate of III [4—6].

Several gas chromatographic procedures have been described for the determination of I in biological fluids utilizing a variety of detection systems [7—18]. For the determination of II or III, two different chromatographic systems were required [5]. Two high-performance liquid chromatographic methods

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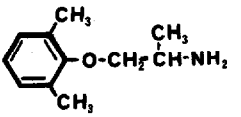
I



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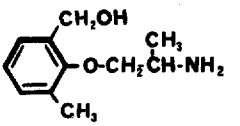

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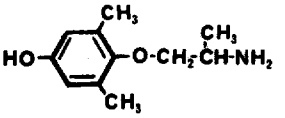

IV

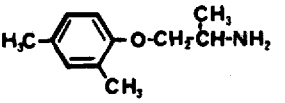

Fig. 1. Structure of mexiletine, its metabolites, and the internal standard. I = mexiletine [1-(2′,6′-dimethylphenoxy)-2-aminopropane], II = hydroxymethyl-mexiletine [1-(2′-hydroxymethyl-6′-methylphenoxy)-2-aminopropane], III = *p*-hydroxy-mexiletine [1-(4′-hydroxy-2′,6′-dimethylphenoxy)-2-aminopropane], and IV = internal standard [1-(2′,4′-dimethylphenoxy)-2-aminopropane].

for the determination of I were published recently utilizing either the UV absorbance of I [19] or of its 2,4-dinitrobenzene derivative [20]. Neither of these methods offers the simultaneous determination of I, II, and III. Pre-column derivatization of primary amines to facilitate their selective detection at low concentrations is a useful technique in liquid chromatography [21, 22]. We report a selective and sensitive method for the simultaneous analysis of I, II, and III following their derivatization with 5-(dimethylamino)-1-naphthalenesulfonyl chloride (Dns chloride). Dns chloride reacts with primary and secondary amine groups, and with phenols at high pH values, yielding highly fluorescent derivatives [21]. This assay was developed to allow the study of the pharmacokinetics of I and its metabolites in patients with renal failure.

EXPERIMENTAL

Reagents and materials

Mexiletine, its metabolites II and III, and the internal standard 1-(2′,4′-dimethylphenoxy)-2-aminopropane (IV), Fig. 1, were gifts from Boehringer Ingelheim (Ridgefield, CT, U.S.A.). Dns chloride was obtained from Sigma (St. Louis, MO, U.S.A.). All other chemicals were analytical grade and the organic solvents for liquid chromatography were distilled in glass.

Individual solutions containing 0.1—3.0 mg (as the free base) of each of I, II, and III per liter of 0.05 *M* hydrochloric acid were prepared. Similar concentrations of these compounds in human serum were also prepared. These serum standards were divided into small aliquots and frozen until needed

for assay. The stock internal standard solution was prepared by dissolving 2 mg of IV in 100 ml of 0.05 *M* hydrochloric acid. The diluted internal standard solution contained 0.2 mg of IV in 100 ml of 0.05 *M* hydrochloric acid.

Procedure

A 100-μl aliquot of the diluted internal standard solution was added to 100 μl of serum, followed by 500 μl of 0.5 *M* carbonate—bicarbonate buffer, pH 9.5. The contents were mixed and extracted with 5 ml of diethyl ether—*n*-butanol (9:1). The organic layer was transferred to another tube and evaporated to dryness under nitrogen. To the residue were added 25 μl of 0.1 *M* sodium bicarbonate solution and 100 μl of a Dns chloride solution in acetone (5 mg per 10 ml). The tube was capped and heated at 70°C for 25 min. The tube was allowed to cool to room temperature, then 100 μl of acetone were added. A 10-μl aliquot of this solution was chromatographed.

Liquid chromatography

A modular liquid chromatograph consisted of a Waters Model 271 microprocessor-controlled gradient system coupled to a filter fluorometer (American Instrument Company, Silver Springs, MD, U.S.A.). The fluorometer had a Corning No. 7-51 primary filter, a Wratten No. 8 secondary filter, and a quartz flow cell, with the relative intensity scale set at 0—10. The stainless-steel column was 250 mm × 4.6 mm I.D., packed with spherical 6-μm Zorbax C8 particles (DuPont Instruments, Wilmington, DE, U.S.A.). The column temperature was ambient. The flow-rate of the mobile phase was 2.8 ml/min. A linear gradient was established over 18 min, starting with 72% methanol in 0.04 *M* ammonium acetate (pH 7.0) and ending with 85% methanol in 0.04 *M* ammonium acetate as the eluent. The concentrations of the compounds in the samples were determined from their peak height ratios relative to the internal standard.

RESULTS AND DISCUSSION

The derivatization of I, II, III, and IV was optimal and reproducible under the conditions described above. The Dns derivatives formed were stable for at least 16 h. The composition and molarity of the eluent were also established. Under these conditions, the retention times for I, II, III, and IV were 9.9, 4.8, 15.9, and 10.9 min, respectively. Fig. 2 shows typical chromatograms obtained from serum samples. Recovery of known concentrations of the three compounds (I, II, and III) in serum ranging from 0.1 to 3.0 mg/l was studied using IV as a reference standard and comparing peak height ratios to those obtained with aqueous standards of I, II, and III. The recovery (mean ± S.D.; $n = 6$) for the concentration range specified was 80.7 ± 3.7% for I (range 76.8—84.2), 85.1 ± 3.7% for II (range 79.9—90.0), and 88.1 ± 3.7% for III (range 84.1—93.8).

Samples of serum standards (100 μl) containing 0.1—3.0 mg of each of I, II, and III per l were analyzed. The relationship between the concentration of each compound and its peak height ratio to IV was found to be linear over the concentration range studied. As little as 100 pg of either compound

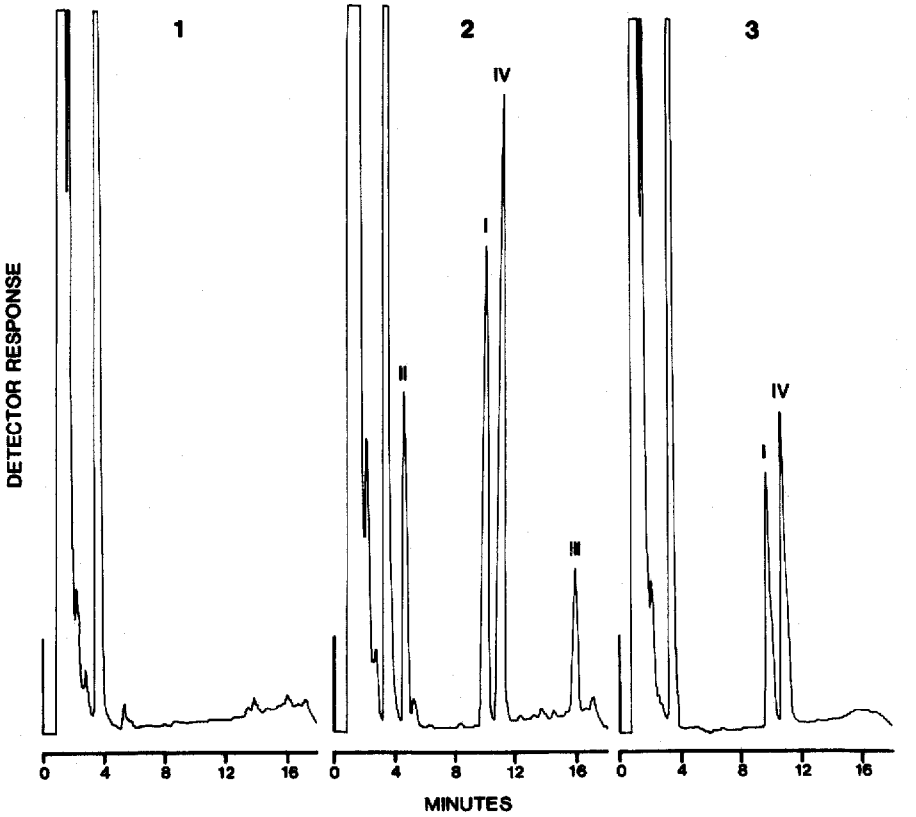

Fig. 2. Chromatograms of serum samples. (1) Patient not receiving mexiletine; (2) standard serum sample containing 2 mg/l of mexiletine (I), 2 mg/l of hydroxymethyl-mexiletine (II) and 1 mg/l of *p*-hydroxy-mexiletine (III); (3) patient receiving mexiletine. The internal standard is IV. Operating conditions: column, Zorbax C8, 250 mm × 4.6 mm I.D.; flow-rate, 2.8 ml/min; eluent A, methanol—0.04 *M* ammonium acetate solution, pH 7.0 (72:28); eluent B, methanol—0.04 *M* ammonium acetate solution (85:15); linear gradient program from 100% A to 100% B over 18 min.

injected on the column could be detected using this procedure. The limit of detection of these compounds was determined after extraction from plasma and was found to be 5 ng/ml.

The precision of this method was determined over several days by analyzing 0.1-ml aliquots of a serum sample containing 2 mg of I and II and 1 mg of III per l. The mean (± S.D.; n = 11) was found to be 2.02 ± 0.15 mg/l for I, 2.11 ± 0.19 mg/l for II, and 1.04 ± 0.11 mg/l for III. These results show a day-to-day precision of 7.4% for I, 9.0% for II, and 10.4% for III.

The proposed method allows for the simultaneous determination of mexiletine and its two major metabolites. If the determination of I alone is desired, an isocratic system can be easily adopted using methanol—0.04 *M* ammonium acetate (80:20) as the eluent, at a flow-rate of 2.5 ml/min where the retention times of I and IV would be 7.9 and 8.9 min, respectively. The use of the gradient was necessary only to reduce the retention time of III. This method also offers the selectivity and sensitivity needed for the determination of I and its metabolites in serum and can be extended to the analysis of these compounds in urine, using the same extraction and derivatization procedures.

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